

The ACT Manual

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Chapter 1. Introduction to ACT

What is ACT?

ACT is a DNA sequence comparison viewer based on [Artemis](#). The name ACT stands for Artemis Comparison Tool. ACT is written in Java, reads EMBL or GENBANK format sequences and feature tables, and can work on sequences of any size.

ACT understands several different comparison file formats. See [the section called *Running a Comparison in Chapter 2*](#) for details. Note that ACT is a comparison viewer only. The comparison information itself must be generated externally. See [the section called *Running a Comparison in Chapter 2*](#) for more details.

For other information see the [ACT web pages](#) or the [Artemis web pages](#).

System Requirements

ACT will run on any machine that has a recent version of Java. This version of ACT requires Java 1.8 at least. Most of the development has been done using JDK v1.8. See [the section called *Getting and Installing ACT*](#) for details on how to get Java.

Getting and Installing ACT

ACT is included in the Artemis tool set package. The most up to date version of ACT is always available from the [ACT web pages](#)

Installing Java

Before installing Artemis/ACT you will need to make sure you have Java installed.

- Linux, UNIX users should get the current Java version from the [Oracle](#).
 - Windows users should get latest stable release from [Java version](#) from Oracle. On some versions of Windows you will need to have administrator privileges for your machine to successfully install Java.
 - For Macintosh users the Java runtime comes packaged with the Artemis software and so does not need to be installed separately.
-

Installation Instructions for UNIX and GNU/Linux

Change directory to the directory you wish to install the Artemis/ACT software in. We will use ~/ in this example and in the next chapter.

Uncompress and untar the `artemis_compiled.tar.gz` file. On UNIX the command is:

```
tar xzf artemis_compiled.tar.gz
```

This will create a directory called `~/artemis` which will contain all the files necessary for running Artemis, ACT and associated tools.

For instructions on how to run ACT on UNIX and GNU/Linux once the archive is unpacked see the [section called *Running ACT on UNIX and GNU/Linux Systems in Chapter 2*](#) .

Installation Instructions for MacOSX

For MacOSX users an archive `artemis.dmg.gz` disk image is provided. Your Mac may uncompress this automatically on download. If not it can be uncompressed using `gunzip`:

```
gunzip artemis.dmg.gz
```

Alternatively on OS X an easier solution is provided by StuffIt Expander. Double-click on any file ending in ".gz" and StuffIt Expander will be launched to uncompress that file.



The uncompressed disk image file "artemis.dmg" can be mounted by double clicking on it. The mounted image, "Artemis_ACT", can then be opened and the software contents displayed by double clicking on it. Open and read the "Readme.txt" file.

Installation Instructions for Windows

On Windows systems with Java (version 1.8 or higher), installing Artemis is as simple as downloading the `artemis-windows.zip` file to an appropriate place (such as the desktop or the Programs folder). Unzip the file using WinZip or equivalent. The unzipped folder will contain the Artemis tool set executable jar files.

For instructions on how to run ACT on Windows once it is unpacked see [the section called *Running ACT on Windows Systems in Chapter 2*](#) .

Concepts

Here are some general concepts about ACT that may make the rest of this manual clearer.

The "Entry"

An "entry" in ACT-speak is not necessarily a complete EMBL or GENBANK entry. In most places in this manual when we refer to an entry we mean a file that contains just the [feature table](#) lines (the FT lines) of an EMBL/GENBANK entry (see [the section called "Tab" Files or "Table" Files](#)). After loading a sequence and opening an entry edit window (see [the section called Open ... in Chapter 2](#)) it is then possible to overlay many feature tables (see [the section called Read An Entry ... in Chapter 3](#)). Each of these feature table files is called an entry by ACT and its features are kept separate from those of other entries.

This meaning of the word "entry" is used by most of the items in the File menu (see [the section called The File Menu in Chapter 3](#)) and by the items in the Entry menu (see [the section called The Entries Menu in Chapter 3](#)).

EMBL/Genbank Features

A "feature" in an EMBL or Genbank file is a region of DNA that has been annotated with a key/type (see [the section called EMBL/Genbank Feature Keys](#)) and zero or more qualifiers (see [the section called EMBL/Genbank Feature Qualifiers](#)). In an EMBL or Genbank formatted file the features of a piece of DNA are listed in the feature table section (see [the section called "Tab" Files or "Table" Files](#)).

EMBL/Genbank Feature Keys

All EMBL and Genbank features have exactly one "key" assigned to them. The key is the type of the feature. Examples include CDS (a CoDing Sequence), intron and misc_feature (MiSCellaneous feature).

The [EBI](#) has a list of [all possible feature keys](#).

EMBL/Genbank Feature Qualifiers

The qualifiers of a feature in an EMBL or Genbank file are the notes and extra information about the feature. For example an exon feature might have a /gene="ratC" qualifier, meaning that the exon feature is part of a gene named ratC.

The [EBI](#) has a list of [all possible feature qualifiers](#).

"Tab" Files or "Table" Files

An EMBL or Genbank file that only contains a feature table (just FT lines, no sequence or header lines) is called a "table" file, or sometimes just a "tab" file because the often has a name like "somefile.tab".

The Active Entries

All entries in ACT are considered to be "active" or "inactive". The overview, DNA view and feature list parts of the main window will only display features from active entries. To find out how to set the active and inactive entries see [the section called *The Entries Menu* in Chapter 3](#).

The Default Entry

Many actions (such as creating features) require an entry to be identified as the source or destination for the action. Some actions, such as "Move Selected Features To ..." in the edit menu, will explicitly ask for an entry, but some assume that the action refers to a "default entry" that was previously set by the user.

The default entry can be set by using the "Set Default Entry ..." menu item in the Entries menu (see [the section called *Set Default Entry* in Chapter 3](#)).

Feature Segments

The term "segment" in the context of a CDS feature means "exon". We use the term "segment", because non-CDS features (such as misc_feature) can have exon-like parts too, but the term "exon" would be inappropriate in that case.

The Selection

In common with applications like word processors and graphics programs, ACT allows the user to "select" the objects that the program will act on. The objects to act on in ACT are features, feature segments or bases. If a feature segment is added to the selection, the feature that contains the segment is implicitly added as well. The current selection can be changed with the Select Menu (see [the section called *The Select Menu* in Chapter 3](#)) or using the mouse (see [the section called *Changing the Selection from a View Window* in Chapter 3](#)).

Feature Colours

Each feature displayed in ACT can be given a colour. The available colours are set in the options file (see [Chapter 5](#)) and are assigned to a feature by adding a `/colour` qualifier (see [the section called *Selected Features in Editor in Chapter 3*](#)). Currently there are two ways of specifying feature colours. The first way uses a single number. For example red is colour 2, so adding `/colour=2` as a feature qualifier will make that feature red. The second way is to specify the red, green and blue components of the colour. Each of the components can take values from 0 to 255, with 255 being the most intense. For example `/colour=255 0 0` is another way to give a feature the colour red. If no `/colour` qualifier is set for a feature a default colour is used (the default colours are also specified in the options file).

Contributions and Suggestions

We welcome contributions to ACT and to Artemis, bug reports from users and suggestions for new features. An email discussion list has been set up for this purpose. To join, send a message to 'artemis - users-join@sanger.ac.uk' with 'subscribe artemis-users' in the body (not the subject). Announcements will also be sent to this list.

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Chapter 2. Starting ACT

Running a Comparison

Before running ACT you will need to obtain a comparison file. ACT supports three different comparison file formats:

- **The output of [BLAST version 2.2.2 or better](#).** The **blastall** command must be run with the **-m 8** flag which generates one line of information per HSP. If [BLAST+](#) is used then the `legacy_blast.pl` PERL script (bundled along with the BLAST+ applications) can be used to generate the tabular format or run it with the **-outfmt 6** flag. For more details see the [BLAST+ manual](#).
- **MEGABLAST output.** ACT can also read the output of [MEGABLAST](#), which is part of the blast distribution.
- **MSPcrunch output.** [MSPcrunch](#) is program for UNIX and GNU/Linux systems which can post-process [BLAST](#) version 1 output into an easier to read format. MSPcrunch much be run with the **-d** flag.

Anthony Underwood and Jonathan Green at the [Health Protection Agency](#) have developed an online tool, [Double ACT](#), to generate ACT comparison files.

Also there is [WebACT](#) which was designed and built by James Abbott and David Aanensen at Imperial College, London and is hosted in the laboratory of Professor Brian Spratt at Imperial College London with funding from The Wellcome Trust.

big_blast.pl

At the Sanger Centre we generally use a perl script called [big_blast.pl](#) to run blast and generate a file of results in MSPcrunch format. To run this script you will need to install a copy of the Pathogen Sequencing Unit [internal perl modules](#). [big_blast.pl](#) can also be used to process blast results after the fact with a command line like this:

big_blast.pl blast_results.out

Note that big_blast.pl has only been tested on UNIX and GNU/Linux machines.

The MSPcrunch Comparison File Format

Here is an example input file generated by MSPcrunch -d.

```
1399 97.00 940 2539 sequence1.dna 1 1596 AF140550.seq
1033 93.00 9041 10501 sequence1.dna 9420 10880 AF140550.seq
828 95.00 6823 7890 sequence1.dna 7211 8276 AF140550.seq
773 94.00 2837 3841 sequence1.dna 2338 3342 AF140550.seq
```

The columns have the following meanings (in order): score, percent identity, match start in the query sequence, match end in the query sequence, query sequence name, subject sequence start, subject sequence end, subject sequence name.

The columns are separated by spaces.

Running ACT on UNIX and GNU/Linux Systems

On Unix and GNU/Linux the easiest way to run the program is to run the script called **act** in the ACT installation directory (see [the section called *Getting and Installing ACT in Chapter 1*](#)) like this:

artemis/act

If all goes well you will be presented with a small window with one menu (the File menu). See [the section called *The ACT Launch Window*](#) for a description of this window.

Alternatively you start ACT with the names of two sequence/EMBL/GENBANK files and a file containing the comparison data eg:

artemis/act sequence1 blast_output.crunch sequence2

If you have more than two sequences (there is no upper limit) they should be listed on the command line separated by the corresponding comparison file:

artemis/act sequence1 seq2_v_seq1.crunch sequence2 seq3_v_seq2.crunch sequence3

See [the section called *UNIX Command Line Arguments for ACT*](#) for a list of the other possible command line arguments. Also to see a summary of the options type:

artemis/act -help

UNIX Command Line Arguments for ACT

As well as the listing file names on the command line, the following switches are available to UNIX users:

-options

This option instructs ACT to read an extra file of options after reading the standard options. (See [the section called *The Options File in Chapter 5*](#) for more about the ACT options file.)

For example `-options ./new_options` will instruct ACT to read `new_options` in the current directory as an options file.

-Xmsn -Xmxn

Use `-Xmsn` to specify the initial size, in bytes, of the memory allocation pool. This value must be a multiple of 1024 greater than 1MB. Append the letter k or K to indicate kilobytes, or m or M to indicate megabytes.

Use `-Xmxn` to specify the maximum size, in bytes, of the memory allocation pool. This value must be a multiple of 1024 greater than 2MB. Append the letter k or K to indicate kilobytes, or m or M to indicate megabytes.

-Dblack_belt_mode=false

If this is set to false then warning messages are kept to a minimum.

-Doffset=10000

This sets the base position that ACT opens at, e.g. 10000.

-Duserplot=pathToFile

This can be used to open userplots from the command line. The paths are comma separated to open multiple plots (-Duserplot='/pathToFile/userPlot1,/pathToFile/userPlot2').

In ACT numbers are used to associate the file with a particular sequence. For example to add a userplot to the second sequence:

act -Duserplot2=/pathToFile/userPlot

-Dloguserplot=pathToFile

This is the same as the above userplot option except that the log transform of the data is plotted.

-Dbam1=pathToFile1 -Dbam2=pathToFile2

This can be used to open BAM/CRAM files and/or VCF/BCF files (see [the section called *Read BAM / CRAM / VCF ... in Chapter 3*](#) for more about the using these file types). This can take a path name to a file or an HTTP address. Numbers are used to associate the file with a particular sequence. For example to add a BAM to the first (top) sequence in ACT:

act -Dbam1=/pathToFile/file.bam

-Dshow_snps

Show SNP marks in BAM/CRAM panels.

-Dshow_snp_plot

Automatically open SNP plots for BAM/CRAM files that are opened.

-Dshow_cov_plot

Automatically open coverage plots for BAM/CRAM files that are opened.

-Dchado="hostname:port/database?username"

This is used to get ACT to look for the database. The address of the database (hostname, port and name) can be conveniently included (e.g. -Dchado="genedb-db.sanger.ac.uk:5432/snapshot?genedb_ro") and these details are then the default database address in the popup login window.

-Dread_only

For a read only chado connection -Dread_only is specified on the command line (e.g. art -Dchado="genedb-db.sanger.ac.uk:5432/snapshot?genedb_ro" -Dread_only).

-Dshow_forward_lines=false

Hide/show forward frame lines.

-Dshow_reverse_lines=false

Hide/show reverse frame lines.

Running ACT on Macintosh Systems

On Macintosh machines ACT can be started by double clicking on the ACT icon. The icon can be added to the dock by dragging it from a 'Finder' window and dropping it onto the position in the dock you want it.

Running ACT on Windows Systems

On systems with Java 1.8 (or higher) installed ACT can be started by double clicking on the act.jar icon.

The ACT Launch Window

This is the first window that opens when you start ACT.

The File Menu

Open ...

If you select this menu item a file requester will be displayed which allows you to open the sequence files and the comparison files. If the files you select are read successfully, a new window will open, which shows the sequences and the comparison. See [Chapter 3](#) to find out how to use the main window. The comparison file is described in [the section called *The MSPcrunch Comparison File Format*](#) .

The "more files ..." button expands the window so that more sequence and comparison files can be read.

Quit

This menu item will close all windows and then exit the program

The Options Menu

Re-read Options

Choosing this menu item will discard the current options settings and then re-read the options file. Note that changing the font size in the file and then selecting this menu item will only change the font size for new windows, not existing windows. Currently some options are unaffected by this menu item. See [the section called *Options File Format* in Chapter 5](#) for more information about options.

Enable Direct Editing

This menu item will toggle "direct editing" option. It is off by default because it can have surprising results unless the user is expecting it. See [the section called "Direct Editing" in Chapter 3](#) for more detail about this.

Genetic Code Tables

These options make all the [NCBI Genetic Codes](#) available. The default setting is the Standard Code. This setting effects the display of start codons (see [the section called Start Codons in Chapter 3](#)) and the "Suspicious Start Codons ..." feature filter (see [the section called Suspicious Start Codons ... in Chapter 3](#)).

Send Searches Via SSH

This is an advanced option for sites that have set up the ability to submit searches via SSH to another machine.

Black Belt Mode

This is an advanced option that can be used to turn off warning message. This options is displayed if the java property (black_belt_mode) is specified on opening up ACT, i.e. `art -Dblack_belt_mode=yes`.

Highlight Active Entry

When this option is on and the "One Line Per Entry" is on (see [the section called One Line Per Entry in Chapter 3](#)) the line that the active entry is on will be highlighted in yellow.

Show Log Window

Show the log of informational messages from ACT. Currently the log window is only used on UNIX and GNU/Linux systems to show the output of external programs. This menu item is only available when running ACT on UNIX or GNU/Linux systems. The logging is controlled by [log4j](#). The `log4j.properties` file (`etc/log4j.properties` in the source distribution) sets the level of logging. This can be used to send the logging information to other places such as a file.

Hide Log Window

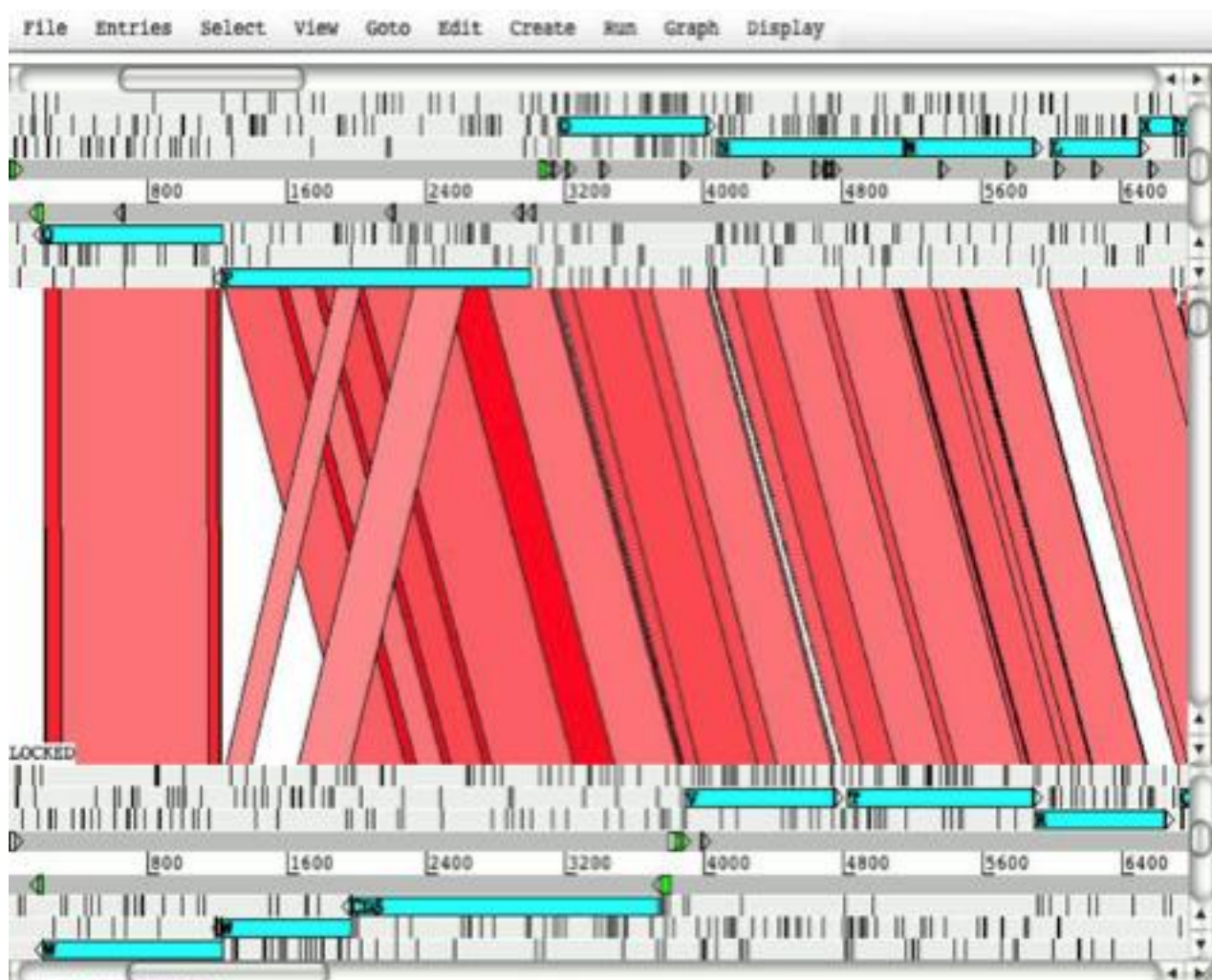
Hide the log of informational messages. This menu item is only available when running ACT on UNIX or GNU/Linux systems.

Chapter 3. The ACT Main Window

This chapter describes the main viewing window of ACT. First is an overview of the window and a description of the menus. The menus are also accessible from the pop-up menu in the view windows (see [the section called *Other Mouse Controlled Functions*](#) and [the section called *The Pop-up Menu*](#) for more).

Overview of the Comparison Window

The following images show a breakdown of the main ACT window taken from a screen shot of a two sequence comparison.



Key:

1. The menus for the main window (described later in this chapter). Each menu contains a sub-menu to control each of the sequences in the comparison. The Display menu (see [the section called *The Display Menu*](#)) is the exception since it controls the overall display. Note that all the menus apart from the File menu and the Display menus can also be accessed via a pop-up menu on the third mouse button. See [the section called *Other Mouse Controlled Functions*](#) and [the section called *The Pop-up Menu*](#) for more.
 2. This a view of the subject sequence of the comparison. See [the section called *The DNA Views*](#) for more information on how to operate this part of the window.
 3. This part of the window shows the comparison between the two sequences. See [the section called *Comparison View*](#) for more information.
 4. The query sequence of the comparison. See [the section called *The DNA Views*](#) for more.
-

The File Menu

Read An Entry ...

Read an entry for this sequence, but keep it separate from the others. See [the section called *The "Entry" in Chapter 1*](#) to see what we mean when we say "entry". The new entry will be marked as active (see [the section called *The Active Entries in Chapter 1*](#)) and will be the new default entry (see [the section called *The Default Entry in Chapter 1*](#)).

This function only reads the feature section of the input file - the sequence (if any) is ignored.

ACT can read these feature files formats:

- [EMBL or GenBank feature tables](#)
 - [GFF files](#)
 - The output of [MSPcrunch](#). **MSPcrunch** must be run with the **-x** or **-d** flags.
 - The output of [blastall](#) version 2.2.2 or better. **blastall** must be run with the **-m 8** flag which generates one line of information per HSP. Note that currently ACT displays each Blast HSP as a separate feature rather than displaying each BLAST hit as a feature.
-

Save Entry

Save the entry to the file it came from, unless the entry has been given a new name, in which case the entry is saved to a file with that name. If the entry has no name, ACT will prompt the user for a new name.

Save All

Save all the entries that have been loaded on top of this sequence.

Write

Amino Acids Of Selected Features

Prompt for a file name and then write the translation of the bases of the selected features to that file. The file is written in FASTA format.

PIR Database Of Selected Features

Prompt for a file name and then write the translation of the bases of the selected features to that file. The file is written in PIR format (similar to FASTA, but with a * as the last line of each record).

Bases Of Selection

Prompt for a file name and then write the bases of the selection to that file in the selected format. If the selection consists of features (rather than a base range) then the bases of each feature will be written to the file as a separate record. If the selection is a range of bases, then those bases will be written.

Upstream Bases Of Selection

Prompt for a number and a file name, then write that many bases upstream of each selected feature to the file in the selected format. For example if the selected feature has a location of "100..200", then

asking for 50 upstream will write the bases in the range 50 to 99. Writing upstream bases of a feature on the complementary strand will work in the expected way.

Downstream Bases Of Selection

Prompt for a number and a file name, then write that many bases downstream of each selected feature to the file in the selected format.

All Bases

Prompt for a file name, then write the complete sequence to that file in the selected format.

Codon Usage of Selected Features

Prompt for a file name, then write a codon usage table for the selected features. The file is written in the same format as the data at [Kazusa codon usage database site](#). In the output file each codon is followed by its occurrence count (per thousand) and its percentage occurrence. (See [the section called Add Usage Plots ...](#) to find out how to plot a usage graph).

Read BAM / CRAM / VCF ...

ACT can read in and visualise BAM, CRAM, VCF and BCF files. These files need to be indexed as described below. Some examples can be found [here](#).

[BAM](#) files need to be sorted and indexed using [SAMtools](#). The index file should be in the same directory as the BAM file. This provides an integrated [BamView](#) panel in ACT, displaying sequence alignment mappings to a reference sequence. Multiple BAM files can be loaded in from here either by selecting each file individually or by selecting a file of path names to the BAM files. The BAM files can be read from a local file system or remotely from an HTTP server.

BamView will look to match the length of the sequence in ACT with the reference sequence lengths in the BAM file header. It will display a warning when it opens if it finds a matching reference sequence (from these lengths) and changes to displaying the reads for that. The reference sequence for the mapped reads can be changed manually in the drop down list in the toolbar at the top of the BamView.

In the case when the reference sequences are concatenated together into one (e.g. in a multiple FASTA sequence) and the sequence length matches the sum of sequence lengths given in the header of the BAM, ACT will try to match the names (e.g. locus _tag or label) of the features (e.g. contig or chromosome) against the reference sequence names in the BAM. It will then adjust the read positions accordingly using the start position of the feature.

When open the BamView can be configured via the popup menu which is activated by clicking on the BamView panel. The 'View' menu allows the reads to be displayed in a number of views: stack, strand-stack, paired-stack, inferred size and coverage.

In ACT the BamView display can be used to calculate the number of reads mapped to the regions covered by selected features. In addition the reads per kilobase per million mapped reads (RPKM) values for selected features can be calculated on the fly. Note this calculation can take a while to complete.

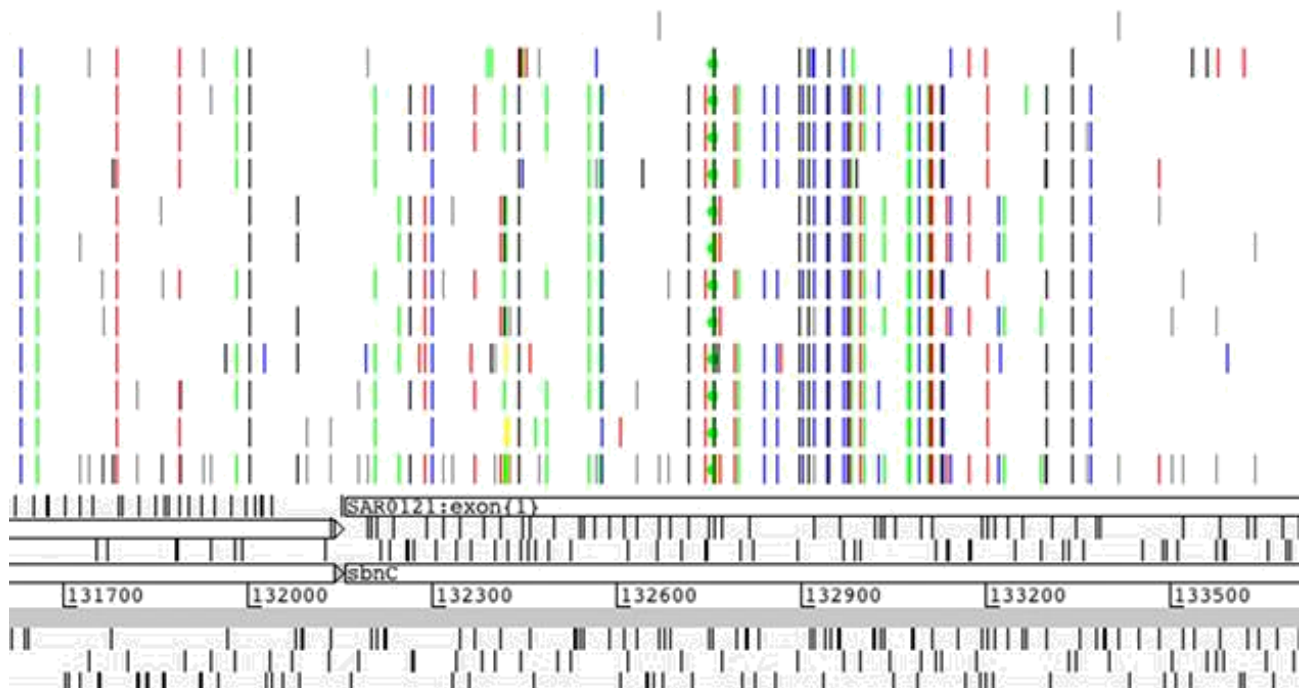
[CRAM](#) files can be loaded in a similar fashion to BAMs. They can be created, sorted and indexed using [SAMtools](#).

Variant Call Format ([VCF](#)) files can also be read. The VCF files need to be compressed and indexed using bgzip and tabix respectively (see the [tabix manual](#) and [download page](#)). The compressed file gets read in (e.g. file.vcf.gz) and below are the commands for generating this from a VCF file:

```
bgzip file.vcf
tabix -p vcf file.vcf.gz
```

Alternatively a Binary VCF ([BCF](#)) can be indexed with BCFtools and read into Artemis or ACT.

As with reading in multiple BAM/CRAM files, it is possible to read a number of (compressed and indexed) VCF files by listing their full paths in a single file. They then get displayed in separate rows in the VCF panel.



For single base changes the colour represents the base it is being changed to, i.e. T black, G blue, A green, C red. There are options available to filter the display by the different types of variants. Right clicking on the VCF panel will display a pop-up menu in which there is a 'Filter...' menu. This opens a

window with check boxes for a number of variant types and properties that can be used to filter on. This can be used to show and hide synonymous, non-synonymous, deletion (grey), insertion (magenta), and multiple allele (orange line with a circle at the top) variants. In this window there is a check box to hide the variants that do not overlap CDS features. There is an option to mark variants that introduce stop codons (into the CDS features) with a circle in the middle of the line that represents the variant. There are also options to filter the variants by various properties such as their quality score (QUAL) or their depth across the samples (DP).

Placing the mouse over a vertical line shows an overview of the variation as a tooltip. Also right clicking over a line then gives an extra option in the pop-up menu to show the details for that variation in a separate window. There are also alternative colouring schemes. It is possible to colour the variants by whether they are synonymous or non-synonymous or by their quality score (the lower the quality the more faded the variant appears).

There is an option to provide an overview of the variant types (e.g. synonymous, non-synonymous, insertion, deletion) for selected features. Also, filtered data can be exported in VCF format, or the reconstructed DNA sequences of variants can be exported in FASTA format for selected features or regions for further analyses. These sequences can be used as input for multiple sequence alignment tools.

Edit In Artemis ...

Open an Artemis edit window for this sequence and features. All changes made in the Artemis window will be immediately visible in the ACT window and vice versa.

See [the Artemis web pages](#) for more about Artemis.

Close

Close this window.

The Entries Menu

The items in this menu are used to change which entry is the default entry and which entries are active (see [the section called *The "Entry" in Chapter 1*](#)). At the bottom of the menu there is a toggle button for each entry which controls whether the entry is active or not.

Here is a description of the other menu items:

Set Name Of Entry

Set the name of an entry chosen from a sub-menu. The name of the entry is used as the name of the file when the entry is saved.

Set Default Entry

Set the default entry by choosing one of the entries from the sub-menu. (See [the section called *The Default Entry in Chapter 1*](#)).

Remove An Entry

Remove an entry from ACT by choosing one of the entries from the sub-menu. The original file that this entry came from (if any) will not be removed.

Remove Active Entries

Remove the entries that are currently active. (See [the section called *The Active Entries in Chapter 1*](#)).

Deactivate All Entries

Choosing this menu item will deactivate all entries. (See [the section called *The Active Entries in Chapter 1*](#)).

The Select Menu

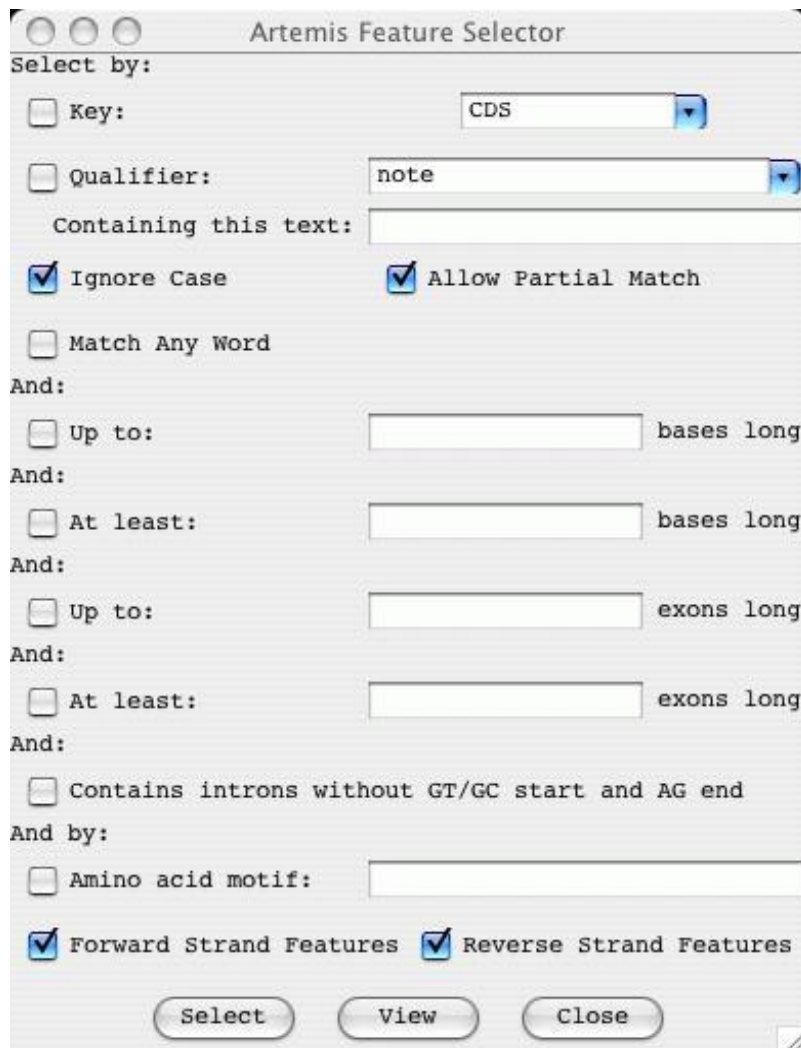
The items in this menu are used to modify the current selection (see [the section called *The Selection in Chapter 1*](#)). Artemis.

Feature Selector ...

Open a new Feature Selector window. This window allows the user to choose which features to select or view based on feature keys (see [the section called EMBL/Genbank Feature Keys in Chapter 1](#)), qualifier values (see [the section called EMBL/Genbank Feature Qualifiers in Chapter 1](#)) and amino acid motifs.

The Select button will set the selection to the contain those features that match the given key, qualifier and amino acid motif combination.

The View button will create a new feature list (see [the section called The Feature List](#)) containing only those features that match the given key, qualifier and amino acid motif combination.



The image shows a screenshot of the 'Artemis Feature Selector' dialog box. The window has a title bar with three standard OS buttons (red, yellow, green) on the left. The main area is titled 'Select by:' and contains several sections of controls. The first section has a 'Key:' dropdown menu set to 'CDS'. Below it is a 'Qualifier:' dropdown menu set to 'note', followed by a text input field labeled 'Containing this text:'. The next section contains two checked checkboxes: 'Ignore Case' and 'Allow Partial Match', and an unchecked checkbox 'Match Any Word'. The following section is labeled 'And:' and contains three pairs of controls: 'Up to:' with a text input and 'bases long', 'At least:' with a text input and 'bases long', and 'Up to:' with a text input and 'exons long'. Below these are two more 'And:' sections, each with an 'At least:' control and a text input followed by 'exons long'. The next section is labeled 'And by:' and contains an unchecked 'Amino acid motif:' text input. At the bottom of the main area are two checked checkboxes: 'Forward Strand Features' and 'Reverse Strand Features'. At the very bottom of the window are three buttons: 'Select', 'View', and 'Close'. A small icon is visible in the bottom right corner of the window.

All

Reset the selection so that nothing is selected then select all the features in the active entries. [shortcut key: A]

All Bases

Reset the selection so that nothing is selected then select all the bases in the sequence.

Select All Features in Non-matching Regions

Select all features that have no corresponding match in ACT. This is used to highlight regions that are different between sets of sequence. It will only take into account matches that have not been filtered out using the score, identity or length cut-off.

None

Clear the selection so that nothing is selected. [shortcut key: N]

By Key

Ask the user for a feature key, reset the selection so that nothing is selected, then select all the features with the key given by the user.

CDS Features

Reset the selection so that nothing is selected, then select all the CDS features that do not have a /pseudo qualifier.

Same Key

Select all the features that have the same key as any of the currently selected features.

Open Reading Frame

Extend the current selection of bases to cover complete open reading frames. Selecting a single base or codon and then choosing this menu item has a similar effect to double clicking the middle button on a base or residue (see [the section called *Changing the Selection from a View Window*](#) for details).

Features Overlapping Selection

Select those (and only those) features that overlap the currently selected range of bases or any of the currently selected features. The current selection will be discarded.

Features Within Selection

Select those (and only those) features that are fully contained by the currently selected range of bases or any of the currently selected features. The current selection will be discarded.

Base Range ...

Ask the user for a range of bases, then select those bases. The range should look something like this: 100-200, complement(100..200), 100.200 or 100..200. If the first number is larger than the second the bases will be selected on the forward strand, otherwise they will be selected on the reverse strand (unless there is a complement around the range, in which case the sense is reversed).

Feature AA Range ...

Ask the user for a range of amino acids in the selected feature and select those bases. The range should look something like this: 100-200, or 100..200.

Toggle Selection

Invert the selection - after choosing this menu item the selection will contain only those features that were not in the selection beforehand.

The View Menu

Selected Features

Open a view window for each selected feature showing it's feature table entry. [shortcut key: V]

Selection

Open a view window that will show the current selection. The window is updated as the selection changes, so it can be left open.

When one feature is selected the window will show the text (EMBL, GenBank or GFF format) of the feature, the base composition, GC percentage, correlation score (see [the section called *Correlation Scores*](#)), and the bases and translation of the sequence of the feature.

When two or more features are selected the window will show the text (EMBL, GenBank or GFF format) of the features, the base composition, average GC percentage, average correlation score, minimum/maximum GC content and minimum/maximum correlation score of the feature sequence.

When a range of bases is selected the window will show the base composition, GC content percentage and the bases and translation of the sequence of the feature.

Search Results

On this sub-menu allows the user to view the results of feature searches that are launched from the run menu in Artemis.

CDS Genes And Products

Pop up a feature list (see [the section called *The Feature List*](#)) of the CDS showing the gene names (from the /gene qualifier) and the product (from the /product qualifier). This list includes pseudo genes.

Feature Filters

Each of the items in this sub-menu each allow the user to view a subset of the active features. An example of a subset is all those features with `misc_feature` as a key. The features are displayed in a new window that contains a menu bar with possible actions to apply to the subset, and feature list (see [the section called *The Feature List*](#)). Most of the possible actions will apply only to the features in the list. For example "Show Overview" in the View menu (see [the section called *Overview*](#)) will include statistics only on the features in the list.

Suspicious Start Codons ...

Show those CDS features that have a suspicious start codon. ie. the first codon of the feature isn't ATG (in eukaryotic mode) or ATG, GTG and TTG (in prokaryotic mode). This function is effected by the setting of the "Eukaryotic Mode" option in the main options menu (see [the section called *Genetic Code Tables in Chapter 2*](#) for more).

Suspicious Stop Codons ...

Show those CDS features that have a suspicious stop codon. i.e. the last codon of the feature isn't one of TAA, TAG or TGA.

Non EMBL Keys ...

Show those features that have a key that isn't valid for EMBL/GenBank entries.

Duplicated Features ...

Show those features that are duplicated (ie. features that have the same key and location as another feature). These sort of duplicates aren't allowed by the EMBL database.

Overlapping CDS Features ...

Show those CDS features that overlap another CDS feature (on either strand).

Features Missing Required Qualifiers ...

Show those features that are missing a qualifier that is required by the EMBL database.

Filter By Key ...

Show those features that have a key chosen by the user.

Selected Features ...

Show the currently selected features in a new feature list. The contents of the list will remain the same even if selection subsequently changes. This is useful for bookmarking a collection of features for later use.

Overview

Open a new window that will show a summary of the active entries and some statistics about the sequence (such as the GC content). [shortcut key: O]

Sequence Statistics

The overview window shows the following statistics about the sequence:

- Number of bases.
 - GC percentage.
 - The number of each nucleotide in the sequence.
 - GC percentage of non-ambiguous bases – i.e. the GC content percentage ignoring bases other than A,T,C and G. This should be the same as the "GC percentage" above.
-

Feature Statistics

The overview window also shows the following statistics about the features in the active entries (if there are any features). Note that the "genes" are the non-pseudo CDS features.

- Number of features in the active entries (see [the section called *The Active Entries in Chapter 1*](#)).

- Gene density - the average number of non-pseudo CDS features per 1000 bases.
 - Average gene length - the average length of non-pseudo CDS features (not including introns).
 - Number of non-spliced genes.
 - Number of spliced genes.
 - Number of pseudo genes (ie. CDS features with a /pseudo qualifier).
 - Protein coding (CDS) features.
 - Protein coding (CDS) bases.
 - Protein coding percentage - ie. the number of coding bases excluding introns.
 - Coding percentage (including introns).
 - A summary of the number of features of each key (type) and their colours.
-

Forward Strand Overview

Open a new window the will show a summary of the features and bases of the forward strand.

Reverse Strand Overview

Open a new window the will show a summary of the features and bases of the reverse strand.

Feature Bases

Create a view window for each selected feature, which shows bases of the feature.

Feature Bases As FASTA

Create a view window for each selected feature, which shows bases of the feature in FASTA format.

Feature Amino Acids

Create a view window for each selected feature, which shows amino acids of the feature.

Feature Amino Acids As FASTA

Create a view window for each selected feature, which shows amino acids of the feature in FASTA format.

Feature Statistics

Show some statistics about each selected feature. On the left on the feature information window is the amino acid composition of the feature. On the right is the codon composition of the feature.

Feature Plots

Open a window for each selected feature that shows a plot of the Kyte-Doolittle Hydrophobicity [short name: `hydrophobicity`], the Hopp-Woods Hydrophilicity [short name: `hydrophilicity`], and an approximation of the GCG Coiled Coils algorithm [short name: `coiled_coil`]. (For more detail about the coiled coils algorithm see "Predicting Coiled Coils from Protein Sequences", Science Vol. 252 page 1162.) [shortcut key: W]

Some general information about graphs and plots in ACT can be found in [the section called *Graphs and Plots*](#). Configuration options for graphs are described in [the section called *Options For Plots and Graphs* in Chapter 5](#).

Adjust panel heights ...

Option in ACT for adjusting the panel heights (BAM, CRAM, VCF, plots, comparisons) by giving them different weights in order to distribute the space between each component.

The Goto Menu

The items in this menu allow the user to navigate around the sequence and features.

Navigator ...

Open a new navigation window. [shortcut key: G]

This window allows the user to perform five different tasks:

1. **Scroll all the views so that a particular base is in the centre of the display .** To use this function, type a base position into the box to the right of the "Goto Base:" label then press the goto button at the bottom of the window. The requested base will be selected and then the overview display and the DNA display will scroll so that the base is as near as possible to the middle of the main window.
2. **Find the next feature that has the given gene name .** To use this function, type a gene name into the box to the right of the "Goto Feature With This Gene Name:" label and then press the goto button. ACT will select the first feature with the given text in any of its qualifiers and will then scroll the display so that feature is in view.
3. **Find the next feature that has a qualifier containing a particular string .** To use this function, type a string into the box to the right of the "Goto Feature With This Qualifier Value:" label and then press the goto button. ACT will select the first feature with the given string in any of its qualifier values (see [the section called EMBL/Genbank Feature Qualifiers in Chapter 1](#)) and will then scroll the display so that feature is in view.
4. **Find the next feature that has a particular key .** To use this function, type a key into the box to the right of the "Goto Feature With This Key:" label and then press the goto button. ACT will select the first feature with the given key and will then scroll the display so that feature is in view.
5. **Find the next occurrence of a particular base pattern in the sequence .** To use this function, type a base pattern into the box to the right of the "Find Base Pattern:" label and then press the goto button. ACT will select the first contiguous group of bases on either strand that match the given base pattern and will then scroll the display so that those bases are in view. Any IUB base code can be used in the pattern, so for example searching for aann₂tt will match any six bases that start with "aa" and ends with "tt". See [Table 3-1](#) for a list of the available base codes.
6. **Find the next occurrence of a particular residue pattern in the sequence .** To use this function, type an amino acid pattern into the box to the right of the "Goto Amino Acid String:" label and then press the goto button. ACT will select the first contiguous group of bases on either strand that translate to the given amino acids and will then scroll the display so that those bases are in view. The letter 'X' can be used as an ambiguity code, hence 'AAXXXDD' will match 'AALRTDD' or 'AATTTDD' etc.

Note that for all the functions above except the first ("Goto Base"), if the "Start search at beginning" option is set or if there is nothing selected the search will start at the beginning of the sequence. Otherwise the search will start at the selected base or feature. This means that the user can step through the matching bases or features by pressing the goto button repeatedly.

If the "Ignore Case" toggle is on (which is the default) Artemis will ignore the difference between upper and lower case letters when searching for a gene name, a qualifier value or a feature key.

The "Allow Substring Matches" toggle affects [2](#) and [3](#). If on ACT will search for qualifier values that contain the given characters. For example searching for the genename CDC will find CDC1, CDC2, ABCDC etc. If the toggle is off ACT will only find exact matches, so searching for the gene CDC will only find features that have `/gene="CDC"` not `/gene="CDC11"`.

Start of Selection

Scroll all the views so that the first base of the selection is as close to the centre as possible. If the a range of bases is selected the views will move to the first base of the range. If one or more features are selected, then the first base of the first selected feature will be centred. Otherwise, if one or more segments (see [the section called *Feature Segments in Chapter 1*](#)) is selected then the first base of the first selected segment will be centred. [shortcut key: control-left]

End of Selection

This does the same as "Goto Start of Selection", but uses the last base of the selected range or the last base of the last selected feature or segment. [shortcut key: control-right]

Feature Start

Scroll the views to the start of the first selected feature.

Feature End

Scroll the views to the end of the first selected feature.

Start of Sequence

Scroll the views so that the start of the sequence is visible. [shortcut key: control-up]

End of Sequence

Scroll the views so that the end of the sequence is visible. [shortcut key: control-down]

Feature Base Position ...

Ask the user for a base position within the first selected feature, then scroll the views so that that base position is centred.

Feature Amino Acid ...

Ask the user for an amino acid position within the first selected feature, then scroll the views so that that position is centred.

The Edit Menu

This menu contains most of the functions that change the entries. Note that the changes will not be saved back to the original files until one of the save functions in the File menu is used.

Undo

This function will undo the last change that was made using the Edit or Create menus. Up to 20 changes can be undone. This menu item is only enabled when there is something to undo. This limit can be changed in the options file (see [the section called *undo_levels* in Chapter 5](#)). [shortcut key: U]

Redo

This function will redo the last undo operation.

Selected Features in Editor

Open an edit window for each selected feature. [shortcut key: E]

Artemis Feature Edit: N

Key: CDS Add Qualifier: note

Location: 4080..5153

Complement Grab Range Remove Range Goto Feature TAT ObjectEdit User Qualifiers

```
/codon_start=1
/transl_table=11
/gene="N"
/product="gpN"
/function="major capsid precursor"
/db_xref="GOA:P25477"
/db_xref="InterPro:IPR006441"
/db_xref="UniProtKB/Swiss-Prot:P25477"
/protein_id="AAD03271.1"
/translation="MRQETRKFKNAYLSRVAELNGIDAGDVSKKFTVEPSVTQTLNMTM
QESSDFLTRINIVPVSEMKGEKIGIGVTGSIASSTDTAGGTERQPKDFSKLASNKYECD
QINFDFYIRYKTLDLWARYQDFQLRIRNAIIKRQSLDFIMAGFNGVKRAETSDRSSNPM
LQDVAVGWLQKYRNEAPARVMSKVTDEEGRTTSEVIRVGKGGDYASLDALVMDATNNLI
EPWYQEDPDLVIVGRQLLADKYFPIVNKEQDNSEMLAADVIISQKRIGNLPAVRVPYF
PADAMLITKLENLSIYYMDDSHRRVIEENPKLDRVENYESMNIIDYVVEDYAAGCLVEKI
KVGDFSTPAKATAEPGA"
```

OK Cancel Apply

From the top down the edit window has these parts:

1. At the top left is a selector for choosing the key of the feature. This only contains a subset of the legal keys. The subset can be changed by changing the `common_keys` option in the options file (see [the section called *common_keys* in Chapter 5](#)).

At the top right of the edit window is a selector for adding a qualifier. For example choosing `note` from the menu will insert `/note=""` into the qualifier edit area.

2. Just below the key and qualifier selector is the location entry field. ACT understands most of the EMBL location syntax, including joins, complements, ranges with non-exact ends (eg. `(100..200) .. >350`) and references to other entries (eg. `join(100..200, SPB23A1:100..200)`).
3. Below the location is a row of buttons:
 - The `Complement` button will complement the contents of the location field.
 - The `Grab Range` button will grab the currently selected range into the location field.



- The Remove Range button will remove the selected bases from the location string. This is normally used to create an intron in a feature.
- Pressing the Goto Feature button has the same effect as the "Start of Selection" item in the "Goto" menu. (See [the section called *Start of Selection*](#) for more).
- The User Qualifiers button opens a tool for maintaining user defined lists of qualifiers (i.e. qualifiers in the form tag = value pairs on separate lines) and the option to read qualifiers from [OBO](#) formatted files or URLs. In the initial screen (see below) you are invited to import your qualifier list from the import options in the "File" menu. These lists can be optionally saved between sessions in the file '.artemis.qualifiers' in the home directory.

When a qualifier list or OBO file has been added then it is possible to search for keywords within a list. The qualifier selected in the drop down list (under the SEARCH button) can then be added to the current feature annotation or added to selected features in ACT. If the keywords text field is left blank then all qualifiers are available from the drop down list of qualifier values.



4. The centre of the edit window contains the qualifier entry section. The qualifiers should be entered in the same way they appear in the feature table part of an EMBL entry, but without the leading FT and spaces.

5. The bottom of the window contains three buttons. The `OK` button will update the feature with the changes that have been made by the user and will then close the edit window. The `Cancel` button will discard the changes and then close the window. The `Apply` will make the changes, but will not close the window. Before any changes are made the location and the qualifiers are checked for formatting errors. Any errors will be brought to the attention of the user through the use of annoying pop-up boxes. No changes will be performed until all errors have been fixed.
-

Subsequence (and Features)

Make a copy (in a new edit window) of the selected bases and the features in that range. Any features that overlap the end of the range will be truncated.

Find Or Replace Qualifier Text

This opens a search window with options to find or replace qualifier text. The search can be restricted to features with a given key and/or it can be restricted to a given qualifier name.

Boolean operators (and/or) can be used in the search. Clicking on the `Show Boolean Search Options` displays 4 options.

1. The `Use boolean operators (and, or, & |)` means that it uses any of these operators that are in the `Find` text field.
2. The `Match any string (i.e. x OR y)` means that the words in the `Find` text field will be separated by an OR condition. So that it finds those features with qualifiers that contain any of the words.
3. The `Match all string (i.e. x AND y)` means that the words in the `Find` text field will be separated by an AND condition. So that it finds those features with qualifiers that contain all of the words.
4. The `No boolean search` option is the default. This means it searches for those features with qualifiers that contain the complete text from the `Find` text field.

In addition selecting the `Duplicate Qualifiers` tab provides options to search for or delete duplicate qualifiers.

Qualifier(s) of Selected Feature

Change ...

This function allows the user to add or change qualifiers on all the selected features in one operation. The main part of the window acts like the qualifier editing field of the feature edit window (see [the section called *Selected Features in Editor*](#)).

Remove ...

This function allows the user to remove all qualifiers with a particular name from all the selected features.

Convert ...

This function allows the user to convert all qualifiers of a particular type to another qualifier for all the selected features.

Selected Feature(s)

Duplicate

Make a copy of each selected feature. Each new feature will be added just after the original in the same entry as the original. [shortcut key: D]

Merge

Create a new feature that contains all the exons and qualifiers of the selected features. The selected features must all have the same key. [shortcut key: M]

Unmerge

If the selection contains exactly two exons and those exons are adjacent in the same feature, split the feature into two pieces between the exons. The original feature is truncated and a new feature is created. The qualifiers of the old feature are copied to new feature.

Unmerge All Segments

All exons in a feature are unmerged.

Delete

Remove each selected feature from its entry. [shortcut key: control-delete]

Delete Exons

Delete the selected exons. The last exon of a feature can't be deleted (delete the whole feature instead).

Remove Introns

Delete the selected introns.

Move Selected Features To

Move the selected features to another entry. Choose the destination entry from the sub-menu.

Copy Selected Features To

Copy the selected features to another entry. Choose the destination entry from the sub-menu.

Trim Selected Features

To Met

For each of the selected features this function will attempt to move the start position to the first ATG in the feature if the feature does not already start on a ATG codon. If there is no ATG in the first thirty percent of the bases of the feature the start position will be unchanged. The search will stop at the end of the first segment of a multi-segment feature.

To Any

This works in the same way as "Trim Selected Features To Met", but will attempt to move the start position of the feature to the first TTG, ATG or GTG in the feature if it does not already start on one of those codons. As above it will only search the first thirty percent of the feature bases and will only search the first segment of a multi-segment feature.

To Next Met

For each of the selected features this function will attempt to move the start position to the next ATG in the feature (the first codon is skipped). If there is no ATG in the first thirty percent of the bases of the feature the start position will be unchanged. The search will stop at the end of the first segment of a multi-segment feature. [shortcut key: T]

To Next Any

This works in the same way as "Trim Selected Features To Next Met", but will attempt to move the start position of the feature to the next TTG, ATG or GTG in the feature (the first codon is skipped). As above it will only search the first thirty percent of the feature bases and will only search the first segment of a multi-segment feature. [shortcut key: Y]

Extend Selected Features

To Previous Stop Codon

Extend each of the selected features which do not start on a stop codon so that each feature starts just after the previous stop codon in this reading frame. [shortcut key: Q]

To Next Stop Codon

Extend each of the selected features which do not end on a stop codon so that each feature ends just before the next stop codon in this reading frame.

To Next Stop Codon and Fix

Same as above but in addition this fixes the stop codons.

Fix Stop Codons

Check and fix the stop codons to all the selected features. For each feature if the last codon is a stop codon, then all is well, nothing further is done to the feature. If the last codon is not a stop codon, but the very next codon is a stop codon, then the end of the feature is moved forward by three bases. If both the last codon and the very next codon after the feature are not stop codons, the feature is selected, an error message is displayed and processing stops immediately.

Automatically Create Gene Names

Ask for a gene name prefix (using a text requester), and then give a unique gene name to each CDS feature in the active entries using that prefix. For example if there are four CDS features with locations: "1..500", "complement(100..600)", "200..700" and "complement(300..800)", entering
SPBC16A3 will give the four features these names: SPBC16A3.01, SPBC16A3.02c, SPBC16A3.03 and SPBC16A3.04c.

Fix Gene Names

For each selected CDS, add the gene name from the CDS to neighbouring/overlapping mRNA, intron, exon, gene, 5'UTR and 3'UTR features. Warn about inconsistencies such as overlapping CDSs.

Bases

Reverse And Complement

Reverse and complement the sequence and all the features in all the entries (active and inactive).

Reverse And Complement Selected Contig

Reverse and complement the sequence and all the features in a selected contig feature. If this option is used in ACT then all the matches within the contig are also reversed. Any matches extending past the

boundary of the contig are deleted. The changes to the comparison file can be saved by right clicking in the comparison window and selecting "Save Comparison File...". However, ideally the comparison between the two sequences should be recalculated.

Delete Selected Bases

Deletes the selected range of bases (if any) from both strands. The deletion will not proceed if the selected range overlaps any features.

Add Bases At Selection

Prompt the user for some bases to insert just before the selected bases. The operation will not proceed if there is no selected range, but bases can be inserted anywhere in the sequence, including inside a feature. The same bases, reversed and complemented, will be inserted at the corresponding place on the opposite strand.

Add Bases From File ...

Prompt the user for the name of a file containing the bases to insert just before the selected bases.

Replace Bases At Selection

Prompt the user for some bases to replace the selected bases.

Contig Reordering ...

Opens a 'Contig Tool' displaying contigs, i.e. with feature keys 'fasta_record' or 'contig'. The former being created automatically for each sequence when a multiple fasta sequence file is read in. The contigs in this tool can then individually be selected and dragged and dropped to another location. In this way the order of contigs and features within a contig can be changed.

If this is used in ACT then the matches are also reordered with respect to the change in the sequence. If a match spans the boundary of a contig that is being moved then if possible it is split. In some situations where there is a match with 'indels' then this is not possible and the match is deleted. The changes to the comparison file can be saved by right clicking in the comparison window and selecting "Save Comparison File...". However, ideally the comparison between the two sequences should be recalculated.

Header Of Default Entry

Open a edit window containing the header of the default entry. Changes made in the edit window will be applied immediately to the entry provided there are no errors in the formatting of the header.

The Create Menu

This menu contains functions for creating new features (see [the section called *EMBL/Genbank Features in Chapter 1*](#)) or entries (see [the section called *The "Entry" in Chapter 1*](#)).

New Feature

Create a new feature in the default entry with a key of "misc_feature" (see [the section called *EMBL/Genbank Feature Keys in Chapter 1*](#)), a location of that spans the whole sequence and which has no qualifiers (see [the section called *EMBL/Genbank Feature Qualifiers in Chapter 1*](#)).

Feature From Base Range

Create a new feature in the default entry with a key of "misc_feature", no qualifiers and a location that exactly matches the selected range of bases. If no bases are selected an error will be reported. [shortcut key: C]

Intergenic Features

Create new features between CDS features in the default entry all with the "misc_feature" key.

Features From Non-matching Regions

Create features in ACT spanning all the regions where a match is not to be found.

New Entry

Create a new entry with no name and no features. The new entry will become the default entry (see [the section called *The Default Entry in Chapter 1*](#)).

Mark Open Reading Frames ...

Create a feature for each "large" open reading frame in the sequence. The default minimum size of a "large" open reading frame can be changed by changing the `minimum_orf_size` option (see [the section called *minimum_orf_size in Chapter 5*](#)). If a codon usage file (see [the section called *Add Usage Plots ...*](#)) has been read each new ORF will have a codon usage score added as a `/score` qualifier. The new features can then be filtered from the display (see "Set Score Cutoffs ..." in [the section called *The Pop-up Menu*](#)).

Mark Empty ORFs ...

Create a feature for each open reading frame that doesn't already contain a feature.

Mark Open Reading Frames In Range ...

Create a feature for each "large" open reading frame in a range of bases. A range must be selected before using this command.

Mark From Pattern ...

Open a text requester to ask the user for a base pattern, then create a feature for each group of bases that matches that pattern. A new entry will be created to hold the features with the name "matches: <pattern>", where <pattern> is the pattern that was entered by the user. Any IUB base code can be used in the pattern, so for example, `aanntt` will match any six bases that start with "aa" and ends with "tt".

Table 3-1. IUB Base Codes

R = A or G	S = G or C	B = C, G or T
Y = C or T	W = A or T	D = A, G or T
K = G or T	N = A, C, G or T	H = A, C or T

M = A or C		V = A, C or G

Mark Ambiguities

Create a new feature for each block of ambiguous bases. The new features will have a key of `misc_feature` and will be created in a new entry called "ambiguous bases".

The Run Menu

This menu is primarily used for running external programs on UNIX. Additionally there are menu options to send sequences from selected features to the [NCBI web BLAST](#), [Pfam](#) and [Rfam](#).

Once configured correctly, running an external program should be as simple as selecting some features of interest, then choosing one of the items from the run menu. When the external program finishes the results can be viewed using the "Search Results" item in the View menu (see [the section called Search Results](#)).

Configuring the Run Menu

To use this feature the `run_blastp`, `run_fasta` etc. scripts that are supplied with ACT will need to be changed to reflect the paths and databases that are configured at each site. Note that the `run` scripts are stored in the `etc/` directory.

Each external program that is listed in the options file (see [the section called feature dna programs in Chapter 5](#) and [the section called feature protein programs in Chapter 5](#)) gets a "run" menu item and a "set options" menu item. For each external program (such as `blastp`) there must be a shell script available that sets any necessary environment variables and then launches the search/analysis program (for `blastp` the script is called `run_blastp`).

Taking `blastp` as an example, this is the sequence of events that occurs when the user selects the "Run blastp on selected features" menu item:

1. ACT creates a new directory in the current directory called `blastp`.
2. A protein FASTA sequence file is written in the new directory for each selected feature. (For a DNA search program such as `blastn` the file will be a DNA FASTA file). The sequence file name will be something like: `blastp/features.tab.seq.00001`.

3. The name of the expected output file is stored in the feature in a qualifier called `/blastp_file`. If the entry is called `features.tab` then the qualifier will be set to something like: `/blastp_file="blastp/features.tab.seq.00001.out"`. Note that because the file name is stored in the entry you will need to save the entry to keep the association between the features and the output files.
4. A file is then written (called something like `blastp/file_of_filenames.1`) that contains the names of all the newly created sequence files in the `blastp` directory.
5. ACT then tries to read the `run_blastp` script from the ACT installation directory. The script is executed like this:

```
run_blastp blastp/file_of_filenames.1 [options]
```

where `[options]` currently must be a single word (normally a database to search). In the case of `blastp/blastn/fasta` etc. the second argument of the script is passed directly to the `blast/fasta` as the database name. For testing purposes it is possible to run `run_blastp` on the command line with the same arguments as above.

`run_blastp` will run `blastp` on each of the sequence files listed in `file_of_filenames.blastp` and save the output in the corresponding `.out` file.

6. If the program is successfully started, control will immediately return to the user. When `run_blastp` finishes a message will be displayed to alert the user.

If necessary, it is possible to exit once ACT indicates that the external program has been started and the entry has been saved. `run_blastp` will keep running in the background.

The Graph Menu

Some general information about the graphs can be found in [the section called *Graphs and Plots*](#). Configuration options for graphs are described in [the section called *Options For Plots and Graphs* in Chapter 5](#).

Hide All Graphs

This item will turn off all the visible graphs.

Add Usage Plots ...

This menu item prompts the user for the name of a file which should contain codon usage data in the same format as the data at [this web site](#). If ACT successfully loads the codon usage file two new plots will be added to the display menu and will be immediately visible. One plot shows the codon scores (in a sliding window) for each of the forward reading frames and the other shows the same thing for the reverse reading frames. [short name: codon_usage]

The graph is calculated using the codon preference statistic from [Gribskov et al. \(Nucl. Acids Res. 12; 539-549 \(1984\)\)](#).

Here is an example usage file:

UUU 32.2 (48423)	UCU 30.5 (45913)	UAU 21.8 (32829)	UGU 8.9 (13371)
UUC 13.0 (19519)	UCC 12.1 (18149)	UAC 11.8 (17721)	UGC 5.6 (8372)
UUA 26.0 (39138)	UCA 17.9 (26850)	UAA 1.3 (1944)	UGA 0.5 (733)
UUG 24.0 (36134)	UCG 8.0 (12055)	UAG 0.5 (705)	UGG 10.9 (16364)
CUU 25.3 (38015)	CCU 21.9 (32964)	CAU 16.3 (24577)	CGU 16.3 (24495)
CUC 7.3 (10922)	CCC 8.4 (12619)	CAC 6.4 (9653)	CGC 6.2 (9316)
CUA 8.6 (12957)	CCA 12.7 (19075)	CAA 27.3 (41066)	CGA 7.9 (11896)
CUG 6.3 (9503)	CCG 4.6 (6910)	CAG 10.9 (16457)	CGG 3.0 (4487)
AUU 35.0 (52636)	ACU 22.9 (34419)	AAU 33.9 (51009)	AGU 14.7 (22108)
AUC 12.6 (19000)	ACC 10.9 (16378)	AAC 17.9 (26895)	AGC 9.2 (13905)
AUA 13.1 (19726)	ACA 13.9 (20898)	AAA 39.3 (59079)	AGA 11.1 (16742)
AUG 20.9 (31376)	ACG 6.5 (9744)	AAG 25.2 (37825)	AGG 5.1 (7615)
GUU 29.3 (44015)	GCU 30.2 (45397)	GAU 38.1 (57240)	GGU 22.0 (33101)
GUC 11.0 (16497)	GCC 11.6 (17518)	GAC 15.8 (23749)	GGC 8.5 (12717)
GUA 12.3 (18451)	GCA 15.7 (23649)	GAA 44.3 (66550)	GGA 15.7 (23623)
GUG 8.3 (12422)	GCG 5.3 (8011)	GAG 21.3 (31979)	GGG 4.3 (6497)

Add User Plot ...

ACT is able to display some types of user data in a graph that looks like the GC content graph (see [the section called *GC Content \(%\)*](#)). This menu item will prompt the user for the name of a data file which should contain one of the following possible graph file formats:

1. one line per base and one or more columns of integer or floating point values per line. The number of lines should match the number of bases in the sequence. ACT will plot each data point for the corresponding base. Each column represents a data set for a line. Example extract:

```
21.4      1910.7
44        1126.1
1911.7    0
0          0
0          1782.0
1937.5    65.4
...
```

2. the first column corresponds to the base position and is an integer. Note to distinguish this format from the previous format the first line of the file must start with a '#'. Line colours can be specified in the header using the keyword colour followed by space separated R:G:B values for each line. The next rows(s) are the data values. Example extract:

```
# BASE VAL1 VAL2 VAL3 VAL4 VAL5 VAL6
# colour 5:150:55 255:0:0 0:255:0 0:0:255 100:100:100 50:150:50
176 2204.8 848.23 0 0 0 536.04
278 804.99 0 837.2 0 681.63 0
452 0 699.98 0 0 0 251.18
553 0 0 0 0 0 52.4
654 0 0 0 334.2 0
686 0 0 652.78 0 0
831 0 0 0 0 67.97
...
```

3. indexed tab delimited file. For this tabix is used (see the [tabix manual](#)) to create an index. This is especially useful for large data sets as it is memory efficient and only reads the data corresponding to the visible region in ACT. The first columns contain the sequence name and base position and this is then followed by the values to be plotted.

For example 'file.plot' is a tab delimited file with column 1 containing the sequence name and column 2 the positions, this is sorted and then indexed with tabix:

```
(grep ^"#" file.plot; grep -v ^"#" file.plot | sort -k1,1 -k2,2n) | bgzip >
sorted.plot.gz ;
tabix -s 1 -b 2 -e 2 sorted.plot.gz
```

Example extract:

```
foo      1      5      5      129      5      5      239
foo      2      1     10     124     12     10     234
foo      3      5     16     129     12     15     229
foo      4      0     23     124     20     20     414
foo      5      5     22     121     28     25     419
foo      6     30     36     124     32     30     412
...
```

4. the next two formats are types of [Wiggle formats](#). The first is variableStep. Note that ACT only supports the colour element in the track line.

```
track type=wiggle_0 color=255,200,0
variableStep chrom=chr19 span=10
310 1
320 12
330 18
340 6
350 5
430 3
440 1
```

Right clicking on the graph and selecting the 'Configure...' option will display the 'Plot style' option for wiggle plots. The plots can be displayed as histograms or as a heat map.

5. the next format supported by ACT is fixedStep and is again a Wiggle format.

```
track type=wiggle_0 name="fixedStep" description="fixedStep format"
visibility=full autoScale=off viewLimits=0:1000 color=0,200,100
maxHeightPixels=100:50:20 graphType=points priority=20
fixedStep chrom=chr19 start=7401 step=300 span=200
1000
```

900
800
700
600
500
400
300
200
100

6. Blast tabular format. The blastall command must be run with the -m 8 flag which generates one line of information per HSP. Alternatively the MSPcrunch file format can be read in as a graph file format. ACT will prompt the user to determine whether it uses the query or subject coordinates to plot the graph.

When a file is prompted for there is an option which if selected will mean the log transform is plotted.

GC Content (%)

Controls whether the GC content plot is visible. This is a graph of the average GC content of a moving window (default size 120 base), across the bases visible in the overview window. [Default: off] [short name: gc_content]

GC Content (%) With 2.5 SD Cutoff

Controls whether the cutoff GC content plot is visible. This is similar to the GC content graph, but the plot is clipped so that the GC content of each algorithm window is shown only when it is more than 2.5 times the standard deviation of the GC content in all the windows. [Default: off] [short name: sd_gc_content]

AG Content (%)

Controls whether the AG content plot is visible. This is a graph of the average AG content of a moving window (default size 120 base), across the bases visible in the overview window. [Default: off] [short name: ag_content]

GC Frame Plot

Controls whether the GC frame plot is visible. This graph is similar to the GC content graph but shows the GC content of the first, second and third position independently. For more information on the algorithm and on how to interpret the result see [this web page](#).

See [Ishikawa, J. and Hotta, K. FEMS Microbiol. Lett. 174:251-253 \(1999\)](#) and [GC frame plot](#) for more information on the algorithm.

[Default: off] [short name: gc_frame]

Correlation Scores

Controls whether the (forward) correlation scores plot is visible. The graph shows the correlation between the amino acid composition of the globular proteins in TREMBL and the composition of the base translation in each of the three reading frames. The green line represents forward frame 1, blue represents frame 2 and red represents frame 3. [Default: off] [short name: correlation_score]

Reverse Correlation Scores

This does the same as "Correlation Scores", but does the calculation on the reverse strand. The green line represents reverse frame 1 (the bottom frame line), blue represents frame 2 and red represents frame 3. [Default: off] [short name: correlation_score]

GC Deviation (G-C)/(G+C)

Controls whether the GC deviation plot is visible. This graph shows the difference between the "G" content of the forward strand and the reverse strand.

See "Asymmetric substitution patterns in the two DNA strands of bacteria" Lobry JR. - [Mol Biol Evol 1996 May;13\(5\):660-5](#).

[Default: off] [short name: gc_deviation]

AT Deviation (A-T)/(A+T)

Controls whether the AT deviation plot is visible. This graph shows the difference between the "A" content of the forward strand and the reverse strand. [Default: off] [short name: at_deviation]

Karlin Signature Difference

This menu item toggles the display of the graph of the dinucleotide absolute relative abundance difference between the whole sequence and a sliding window.

For details of the algorithm see "Global dinucleotide signatures and analysis of genomic heterogeneity" Samuel Karlin - [Current Opinion in Microbiology 1998, 1:598-610](#).

[Default: off] [short name: karlin_sig]

Cumulative AT Skew and Cumulative GC Skew

AT skew is calculated as $([A]-[T])/([A]+[T])$, where [A] and [T] are the counts of these bases in the window. Grigoriev A (1999) [Strand-specific compositional asymmetries in double-stranded DNA viruses. Virus Research 60, 1-19](#).

Positional Asymmetry

Shulman MJ, Steinberg CM, Westmoreland N (1981) [The coding function of nucleotide sequences can be discerned by statistical analysis. J Theor Biol 88:409-20](#).

Informational Entropy

Konopka Andrzej (1984) [Is the information content of DNA evolutionarily significant? J Theor Biol 107:697-704](#). Informational entropy is calculated from a table of overlapping DNA triplet frequencies, using equation 1 in the above reference. The use of overlapping triplets smooths the frame effect.

Scaled Chi Square

Shields DC, Sharp PM (1987) [Synonymous codon usage in Bacillus subtilis reflects both translational selection and mutational biases. Nucleic Acids Res 15:8023-40](#).

Mutational Response Index

Gatherer D, McEwan NR (1997) [Small regions of preferential codon usage and their effect on overall codon bias--the case of the plp gene. Biochem Mol Biol Int 43:107-14.](#)

Effective Codon Number

Wright F (1990) [The 'effective number of codons' used in a gene. Gene 87:23-9](#), and Fuglsang A (2004) [The 'effective number of codons' revisited. Biochem Biophys Res Commun. May 7;317\(3\):957-64.](#)

Intrinsic Codon Deviation Index

Freire-Picos MA, Gonzalez-Siso MI, Rodriguez-Belmonte E, Rodriguez-Torres AM, Ramil E, Cerdan ME (1994) [Codon usage in Kluyveromyces lactis and in yeast cytochrome c-encoding genes. Gene 139:43-9.](#)

The Display Menu

The items in this menu control the global appearance of the display.

Hide Frame Lines

Selecting this menu item will turn off the frame lines of the query and subject views. The frame lines can be turned on selectively using the pop up menu in the view (see [the section called The Pop-up Menu](#)).

Show Frame Lines

Selecting this menu item will turn on the frame lines of the query and subject views.

The DNA Views

The two sequence views in ACT show the forward and reverse strands of the sequence and a representation of the three translation frames in each direction. The top view shows the subject sequence of the comparison (see [2](#)), the bottom view shows the query sequence (see [4](#)). The horizontal scrollbar controls which part of the sequence is currently visible. The scroll bar at the left controls the zoom level. By default the view shows only the stop codons in each translation frame, but if the view is zoomed in it will show the complete six frame translation.

See also [the section called *Overview of the Comparison Window*](#).

Changing the Selection from a View Window

To select a feature just click on it with the first mouse button. This will unselect anything that is currently selected. To add a feature to the selection rather than replacing the current selection, hold the `shift` key while clicking. A single feature can be removed from the selection in the same way, because shift-clicking acts as a toggle. The situation is made slightly more complicated by the existence of spliced genes. When a feature segment (exon) is added to the selection the feature that contains the segment is implicitly added as well. When the last segment of a feature is removed from the selection, the feature is implicitly removed.

A single base or amino acid can be selected simply by clicking on it. A range of bases can be added by clicking on the base at one end of the range then shift-click on the base at the other end of the range. Alternatively you can drag out a range of bases: click on a base then hold the mouse button down and move to the other end. To select a complete open reading frame (ORF), double click the middle mouse button (see [the section called *Notes on Using The Mouse*](#)) anywhere inside the ORF (see [the section called *Open Reading Frame*](#) for another way to select an ORF).

See [the section called *The Selection in Chapter 1*](#) for more about the selection.

Other Mouse Controlled Functions

Double clicking on a feature with the first mouse button causes the view to centre itself on that feature. Similarly, double clicking the first mouse button on a base or amino acid will centre the view on that base/amino acid.

A double click of the middle mouse button on a feature will open an edit window for that feature. This is the same as clicking once and then choosing the Edit Selected Features menu item (see [the section called *Selected Features in Editor*](#)).

The third button activates a pop-up menu with functions specific to this view. See [the section called *The Pop-up Menu*](#) for more.

The Pop-up Menu

The pop-up menu is activated by pressing the third mouse button (see [the section called *Notes on Using The Mouse*](#)) on a feature view. The menu is split into three sections. The top contains functions that will act on the current view. The middle contains shortcuts to some of the main window menus. The bottom contains four toggle buttons which influence the appearance of the view.

Pop-up Menu Functions

Note that not all of these functions are available all the time (the first two are only shown when there are some selected features).

- **Raise Selected Features.** Raise the selected features so that they appear in front of all other features.
 - **Lower Selected Features.** Make the selected features go behind all the other features.
 - **Smallest Features In Front.** Sort the visible features so that the smallest features appear in front of the larger ones. This is only necessary when the user has manually rearranged the features using "Raise Selected Features" or "Lower Selected Features".
 - **Zoom to Selection.** Scroll and scale the display so that the current selection is centred and full width.
 - **Select Visible Range.** Select the currently visible bases on the forward strand.
 - **Select Visible Features.** Select those (and only those) features that currently visible in this view. Any features that are off screen or have been filtered out with the "Set Score Cutoffs ..." control.
 - **Set Score Cutoffs ...** The score cutoffs panel allows the user to filter the features of the active entries so that features with low or high scores are not shown. The "score" of a feature is the value of the `/score` qualifier and should be a number from 0 to 100. The cutoffs window has two sliders. Any feature that has a score less than the value of the top controller or more than the value of the bottom controller will not be shown. Features with no `/score` qualifier will always be shown.
-

Toggle Buttons

Feature Labels

This toggle button controls whether the feature labels are displayed on their own line (when the toggle is on) or on the top of the features (when the toggle is off). The default setting for this toggle can be set in the options file (see [the section called *feature_labels* in Chapter 5](#)).

One Line Per Entry

This toggle controls whether the entries are shown in the context of the three frame translation or one entry per line on screen. In the first case the entries will be overlaid, in second case they will be shown in parallel. The default setting for this toggle can be set in the options file (see [the section called *one line per entry* in Chapter 5](#)).

Forward Frame Lines

This toggle button controls whether or not to show the 3 frame translation of the forward sequence.

Reverse Frame Lines

This toggle button controls whether or not to show the 3 frame translation of the reverse sequence.

Start Codons

Toggle the display of start codons in the view. See [the section called *Genetic Code Tables* in Chapter 2](#) to find out how to change which start codons to use.

Stop Codons

Toggle the display of stop codons in the view.

Feature Arrows

Toggle the display of directional arrows on each feature. The default setting for this toggle can be set in the options file (see [the section called *draw feature arrows* in Chapter 5](#)).

Feature Borders

Toggle the display of black borders around each feature. The default setting for this toggle can be set in the options file (see [the section called *draw feature borders* in Chapter 5](#)).

All Features On Frame Lines

Normally non-protein features are drawn on the DNA lines. This toggle allows the user to force all features to be drawn on the frame lines, which can sometimes improve readability. The default setting for this toggle can be set in the options file (see [the section called *features on frame lines* in Chapter 5](#)).

Flip Display

If selected the sequence and features will be drawn on screen as if they are reverse complemented with the first base to the right of the screen.

Colourise Bases

This toggle turn base colouring on or off. (Note that this feature is completely useless, it exists for amusement only).

Scrolling The View

Moving the horizontal scrollbar will change the part of the sequence that is visible. The position of the view can also be changed by using the Goto menu (see [the section called *The Goto Menu*](#)), by double clicking the first mouse button on a feature in one of the sequence views by double clicking on a base or amino acid.

Changing The Scale

The vertical scrollbar at the right edge of the view controls the scale - moving the scrollbar up will zoom in and moving the scrollbar down will zoom out. When the scrollbar is at the top (at it's most "zoomed-in" position), the complete six frame translation is shown. Otherwise only the stop codons are shown.

"Direct Editing"

The direct editing option (See [the section called *Enable Direct Editing* in Chapter 2](#)) enables the user to change the start or end position of a segment by dragging it around with the mouse. This works best in the DNA view window. As an example, to move the start position, click the mouse button on the first

base of the feature or exon, hold the button down, move the mouse pointer to the desired position, then release the button.

Comparison View

This part of the window shows the comparison between two sequences. Each red/pink box corresponds to single match, with red representing a good match and white/pale pink a lower scoring match. This view cannot be scrolled directly, instead it tracks the movement of the subject and query sequence views (see [the section called *The DNA Views*](#)).

See [3](#) to see where this view fits in the main window.

Controls

The vertical scrollbar at the right of the view sets the minimum size (in bases on the subject sequence) of the matches that are displayed. The default is 1, so when started ACT will show all matches.

Clicking the left button on a match will highlight the match. Double clicking the mouse on one of the matches will cause the sequence views to align around that match.

Clicking the middle mouse button toggles the locking of the two sequences that are being compared by this comparison view. When locked the sequences will scroll in parallel. When unlocked the sequences can be scrolled independently. (See [the section called *Notes on Using The Mouse*](#) if you do not have a middle mouse button)

The Comparison View Pop-up Menu

The pop-up menu is activated by pressing the third mouse button within the comparison view. The menu items are as follows:

- **View Selected Matches.** Creates a new window which contains a list of currently selected (ie. yellow) matches. Clicking on a match in the list will centre the comparison view on that match.
- **Flip Query Sequence.** Flips the query sequence display so that it appears to be reverse complemented. (See [the section called *Flip Display*](#) .)
- **Flip Subject Sequence.** Flips the subject sequence display so that it appears to be reverse complemented. (See [the section called *Flip Display*](#) .)
- **Lock Sequences.** Lock the two sequences so that they scroll together.

- **Unlock Sequences.** Allow the two sequences to scroll independently.
 - **Set Score Cutoffs ...** Pop-up a gadget that allows the matches to be filtered on "score", generally the BLAST score .
 - **Set Percent ID Cutoffs ...** Pop-up a gadget that allows the matches to be filtered on the percentage identity.
 - **Offer To RevComp.** When this option is "on", double clicking on a reverse complemented match will cause ACT to ask the user whether to flip the query sequence before centering on the match. If "off" the match will just be centred.
 - **Ignore Self Matches.** When this option is "on", matches that have the same start and end positions on both the subject and query sequences will not be shown.
-

The Feature List

The feature list is bit at the bottom of the main window of Artemis (see [the section called *Overview of the Comparison Window*](#)). The same component is used by the two items in the view menus that show a sub-set of components (see [the section called *CDS Genes And Products*](#) and [the section called *Feature Filters*](#)). The list contains information about each feature in each active entry (see [the section called *The Active Entries in Chapter 1*](#)), one per line.

The lines show this information about the feature: the colour (see [the section called *Feature Colours in Chapter 1*](#)), the key, start base, end base, the note field (if any) and then the remaining qualifiers in EMBL/GENBANK format. If the correlation scores option is on then the scores will be shown just before the note in each CDS line.

Changing the Selection from the Feature List

To select a feature from the list just click on it with the first mouse button. This will unselect anything that is currently selected. To add a feature to the selection rather than replacing the current selection, hold the `shift` key while clicking. A single feature can be removed from the selection in the same way, because shift-clicking acts as a toggle.

See [the section called *The Selection in Chapter 1*](#) for more about the selection.

Other Mouse Controlled Functions

Double clicking on a feature with the first mouse button causes both views and the feature list to centre themselves on that feature.

A double click of the middle mouse button on a feature will open an edit window for that feature. This is the same as clicking once and then choosing the Edit Selected Features menu item (see [the section called *Selected Features in Editor*](#)).

The Pop-up Menu

The pop-up menu is activated by pressing the third mouse button (see [the section called *Notes on Using The Mouse*](#)) anywhere on the feature list. The menu contains shortcuts to some of the main window menus and three toggle buttons:

Save List to File

Save the list of features to a file.

Show Selected Qualifier

A qualifier can be selected to be displayed in the feature list.

Show Correlation Scores

If this toggle is on the correlation scores for each feature will be show between the end base and the note.

Show Gene Names

This button controls whether the key or gene name is shown for each feature. The "gene name" is actually the `/label` qualifier of the feature. If there is no `/label` then the `/gene` qualifier is used. If neither of the qualifiers exists then the key is used.

Show Qualifiers

If this option is on the feature qualifiers will be displayed after the note.

Show Product

This button controls whether the `/note` or `/product` is shown for each feature. [default: `/note`]

Scrolling The List

The scrollbar on the right of the list controls which part of the list of active features is visible. The list will also move if the user double clicks on a feature in overview or DNA view areas, in which case the list will scroll to that feature.

Graphs and Plots

The DNA plots are accessed from the Graphs(top) and Graphs(bottom) menus (see [the section called *The Display Menu*](#)). When a graph is turned on it is locked to the overview window, so it will follow the overview window when the overview is scrolled. It will also automatically change the viewing scale to match the scale of the overview window. The vertical scrollbar to the right of the graph controls the window size of algorithm.

The feature plots window (see [the section called *Feature Plots*](#)) has a horizontal scroll bar that sets the position of the plot in the feature. As with the DNA plots, the vertical scrollbar to the right of the graph controls the window size of algorithm.

Note that clicking the left mouse button on the plot will show the base or residue at that position. Clicking the right mouse button will pop-up a menu. The menu contains a toggle which controls whether the graph is scaled and some menu items which allow the maximum window size to be set.

Notes on Using The Mouse

ACT makes use of all three buttons of the mouse under X, but other windowing systems often have less than three buttons. To make life easier when running ACT on those systems, there are some alternative key bindings.

On Windows pressing the `alt` or `meta` key and the first mouse button will give the same effect as pressing mouse button three (usually a pop-up menu). Pressing the `control` key and the first mouse button will do the same thing as mouse button two.

On Macintosh systems pressing the `control` key and the mouse button will give the same effect as pressing mouse button three (usually a pop-up menu). Pressing the `alt` key and the mouse button will do the same thing as mouse button two.

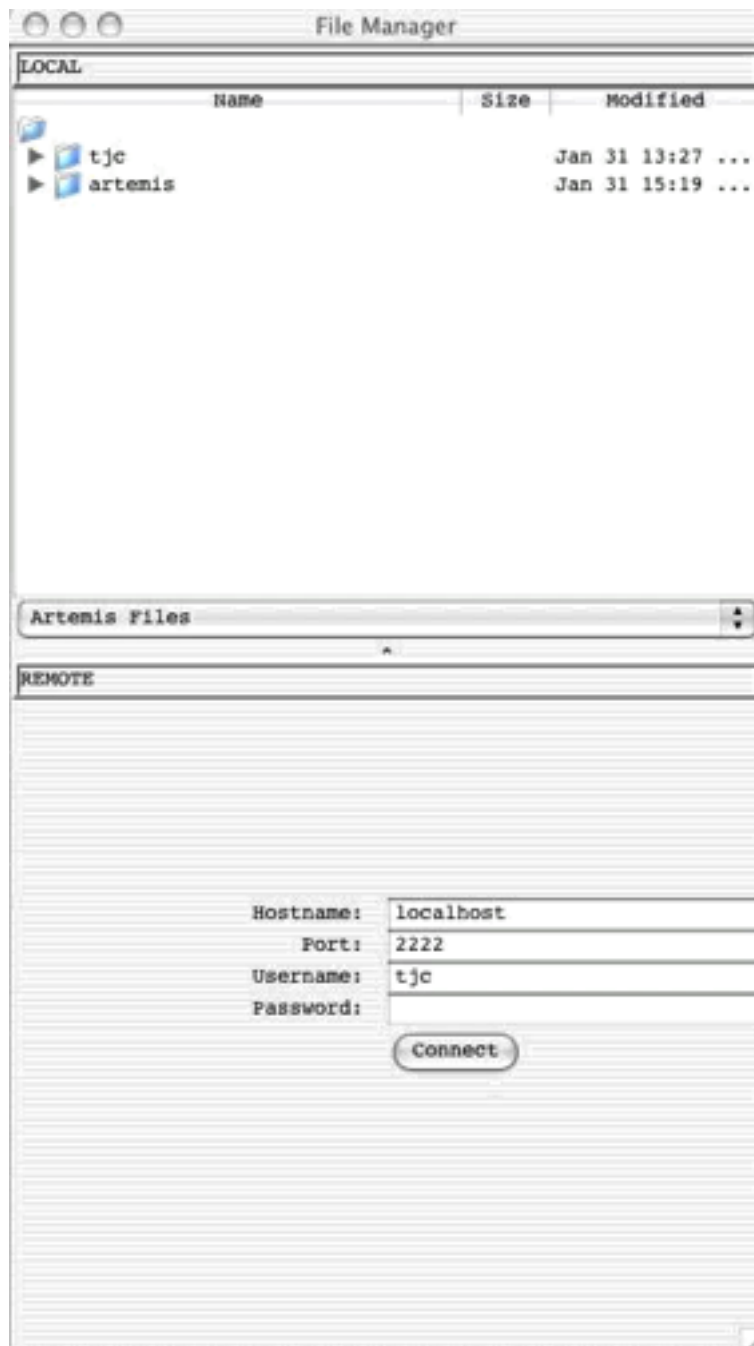
Chapter 4. Secure Shell (SSH) Plugin To ACT Overview of the SSH Plugin

This is a plug-in to ACT that enables you to view and transfer files from a local machine (e.g. PC/MacOSX/UNIX) to another remote file system (i.e. any other file system that you have ssh access to). The main areas where this is useful are:

- file management for both local and remote files. Files can be transferred by dragging and dropping between file systems. Multiple files can be selected and transferred in this way.
 - launching Artemis and ACT with sequences and tab files from files on the remote machines.
 - running BLAST and Fasta searches can be sent from the local machine that is running ACT and run on the remote machine. To do this the software (BLAST and Fasta) need to be set up on the remote side (see [the section called *Configuring Searches to be run via SSH*](#)).
-

Login In

From the ACT launch window go to the 'File' menu and select 'Open SSH File Manager'. A file manager window will then open with a local file manager at the top. Underneath this is a remote file manager with a section to enter login details.



If you can connect directly to the remote machine give the hostname, username and password. Alternatively if you are using a SSH tunnel (see [the section called *Setting up a SSH tunnel*](#)) then use localhost and the appropriate port number.

Setting up a SSH tunnel

The tunneling capability of SSH Secure Shell allows people to access their email, intraweb pages and files securely even when working away from the office. This can be used with ACT and this section provides some examples of setting up tunnelling. It may be necessary to consult your friendly system administrator about the details on how best to do this.

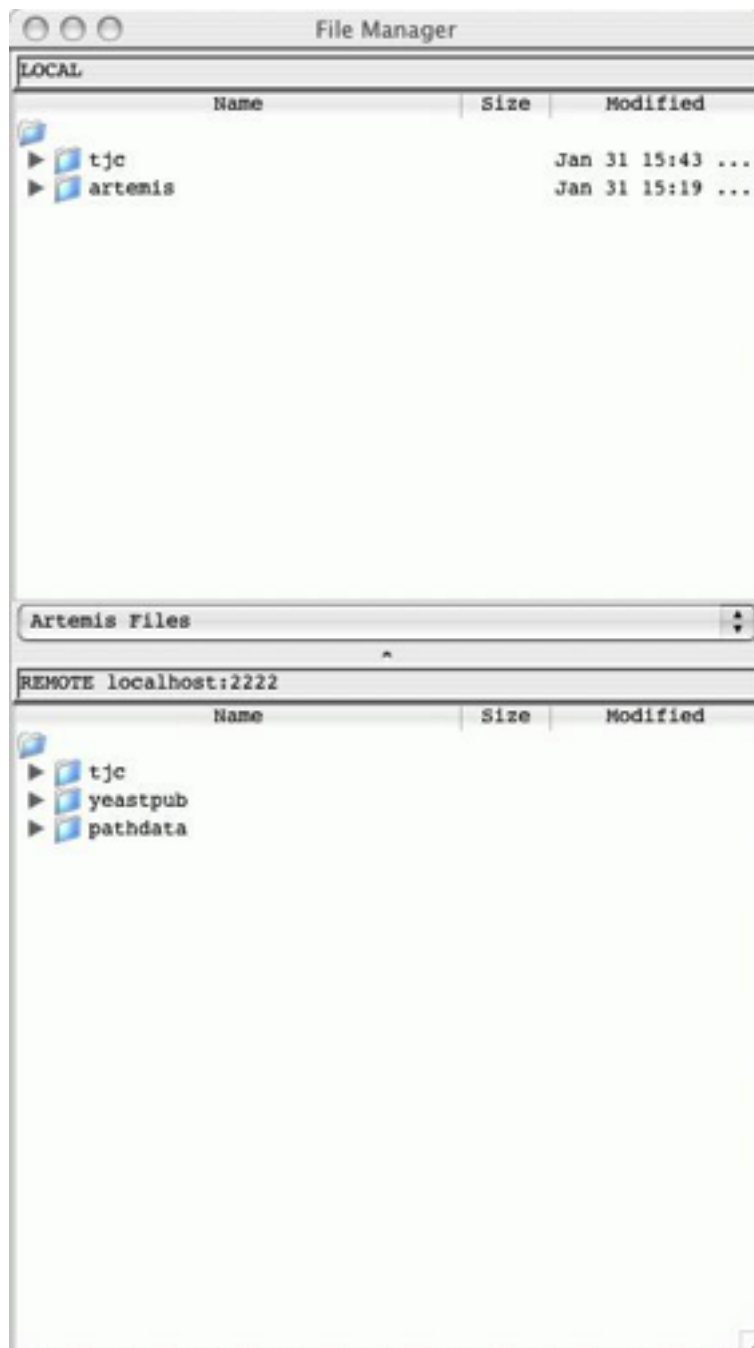
On *UNIX* the ssh configuration can be set in the file `~/.ssh/config`. This contains the information to set up a tunnel that looks like this:

```
Host *.sanger.ac.uk
    LocalForward 2222 machine.sanger.ac.uk:22
    User tjc
```

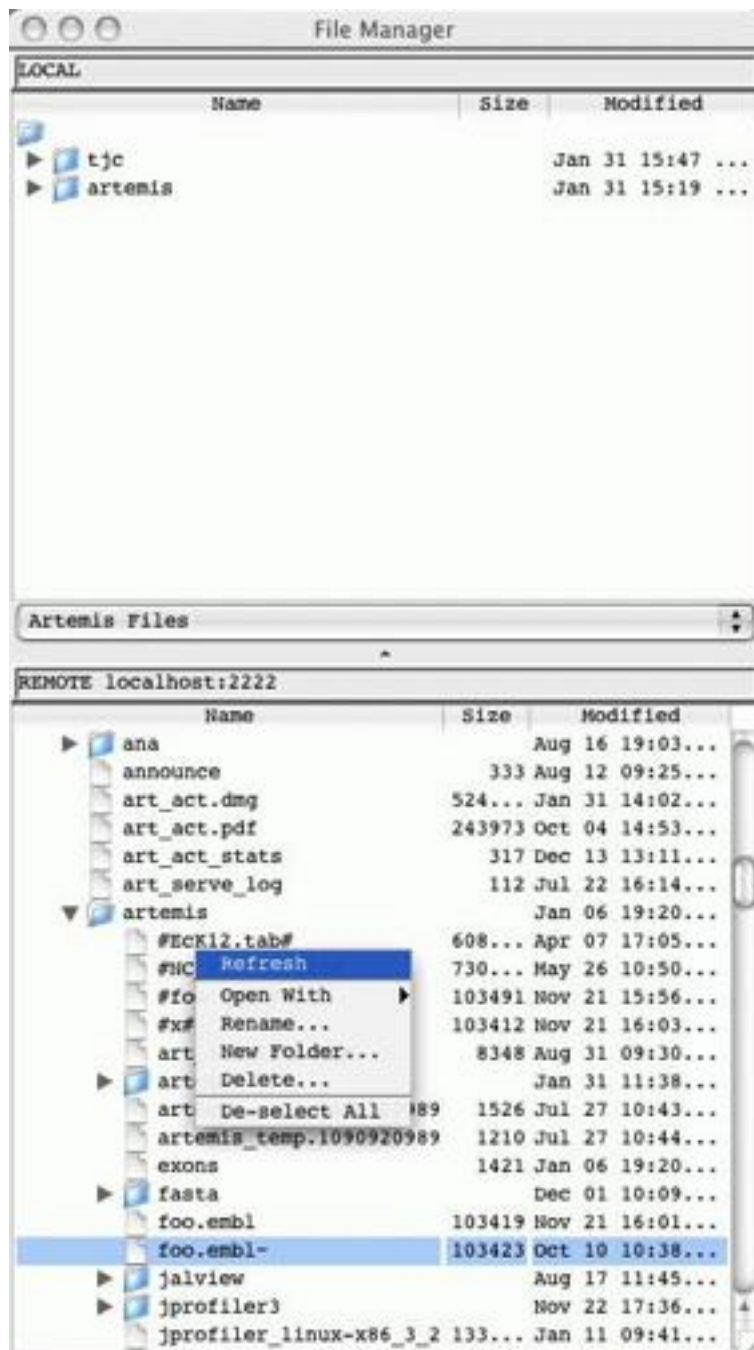
Once this configuration file is in place you can login from a UNIX window to set up a tunnel.

On *Windows* you can use Putty, see [the section called *Using Putty to Set Up A Tunnel*](#).

When the tunnel has been established you can start Artemis and enter 'localhost' into the Hostname field in the login window and the port number (e.g. 2222 in the above example).



The file manager can be used to drag and drop files between each file system. If you right click on a selected file this will provide you with a pop-up menu (see below) enabling you to delete, rename and open files.



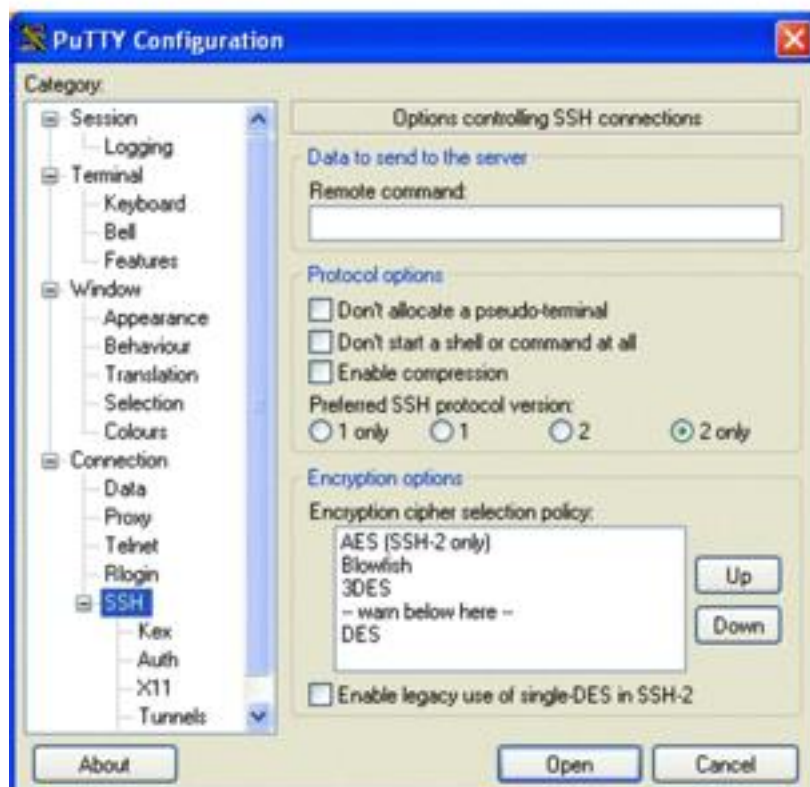
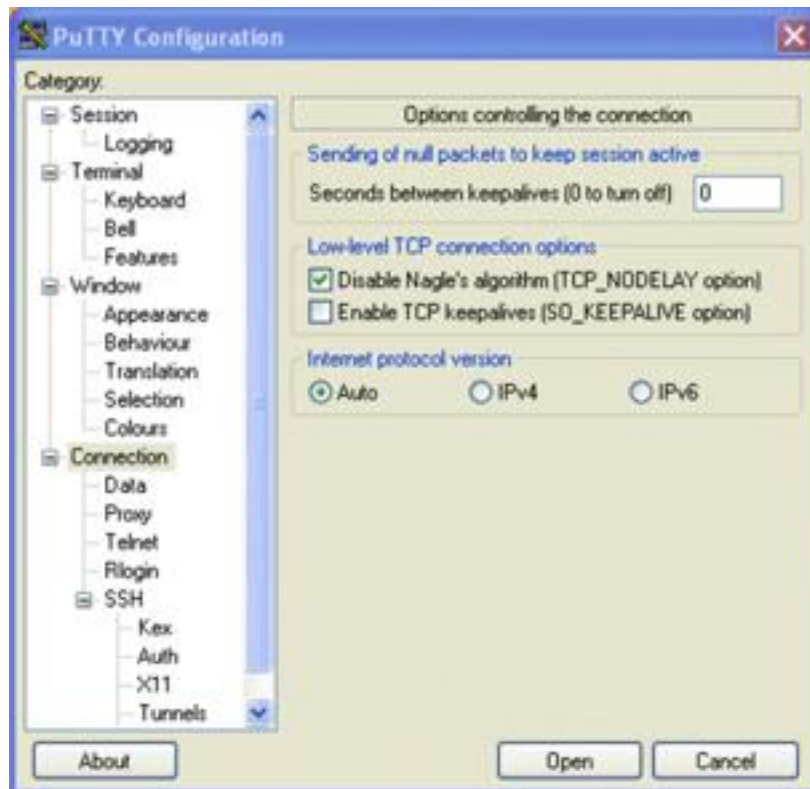
Files on the remote file system can be double clicked on and opened in Artemis (in the same way they can be opened from the local file manager). Tab files can be read in by dragging them from the remote file manager and dropping them into the Artemis window.

Note for ACT the sequence filename can be dragged from the file manager into the file selection box (if the files are not found locally the are then located on the server). The tab files in ACT can be dragged from the file manager and dropped on the relevant sequence display.

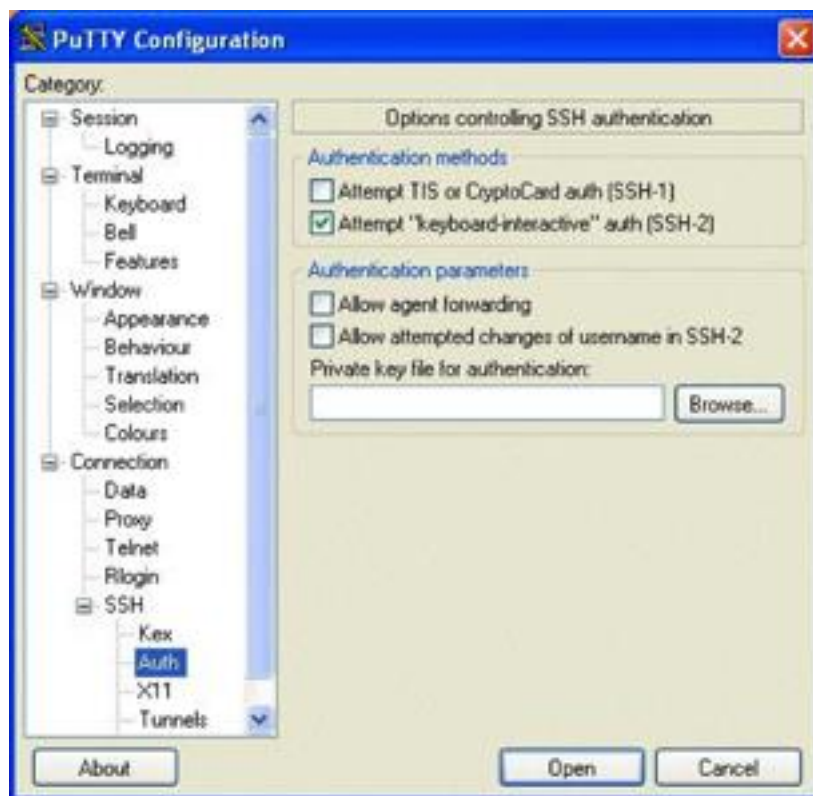
When files are saved, if the entry originated from the remote file system then this is saved locally in the current working directory and to the remote file as well.

Using Putty to Set Up A Tunnel

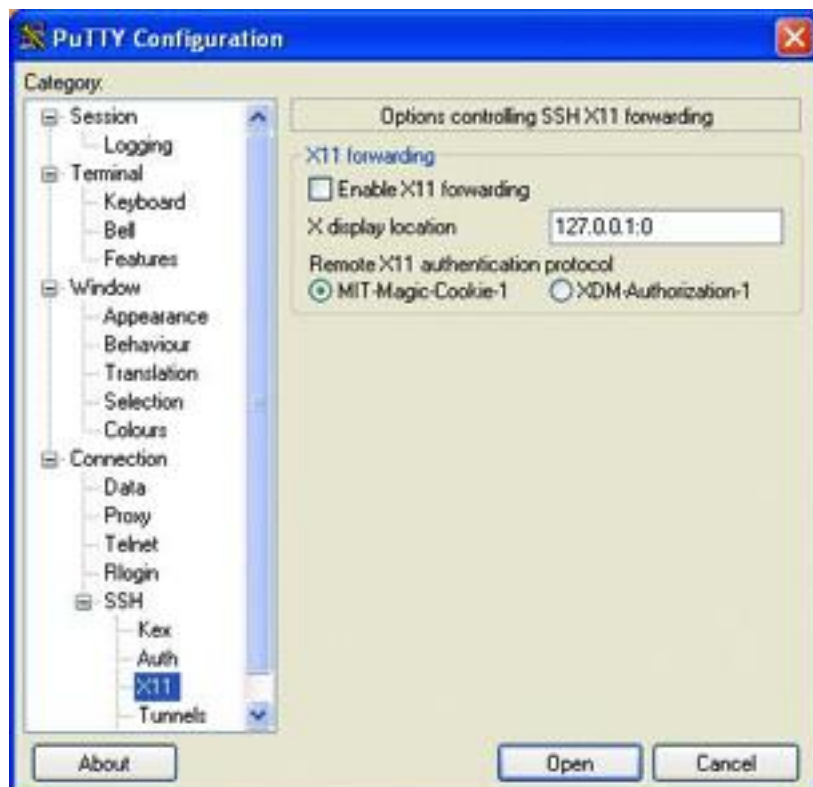
On *Windows* you can use [Putty](#) to set up a ssh tunnel. Below are settings that can be used in Putty to set up a SSH tunnel.



SSH 2 only is probably recommended here.



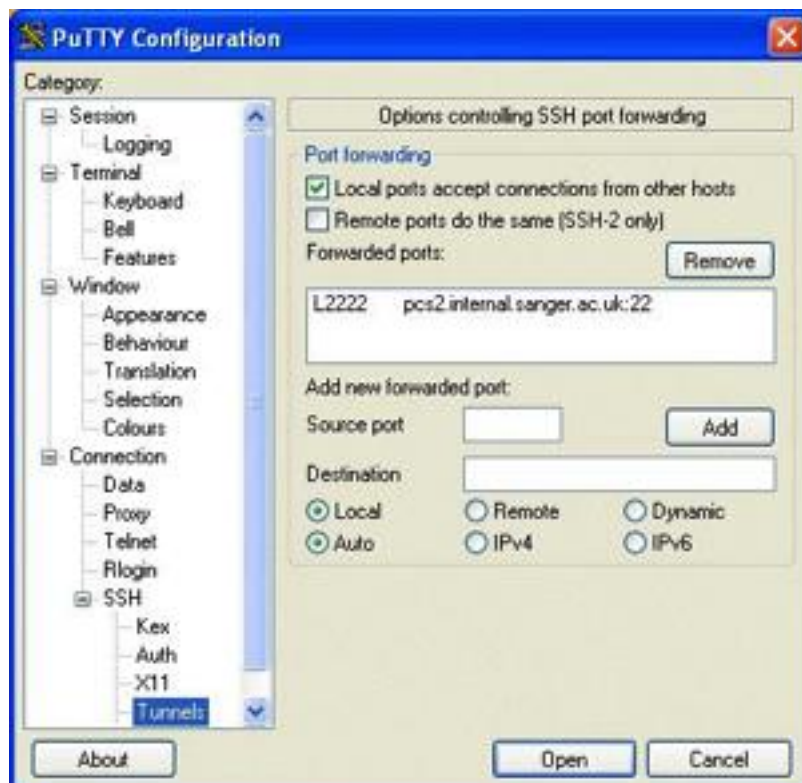
X11 forwarding is not necessary



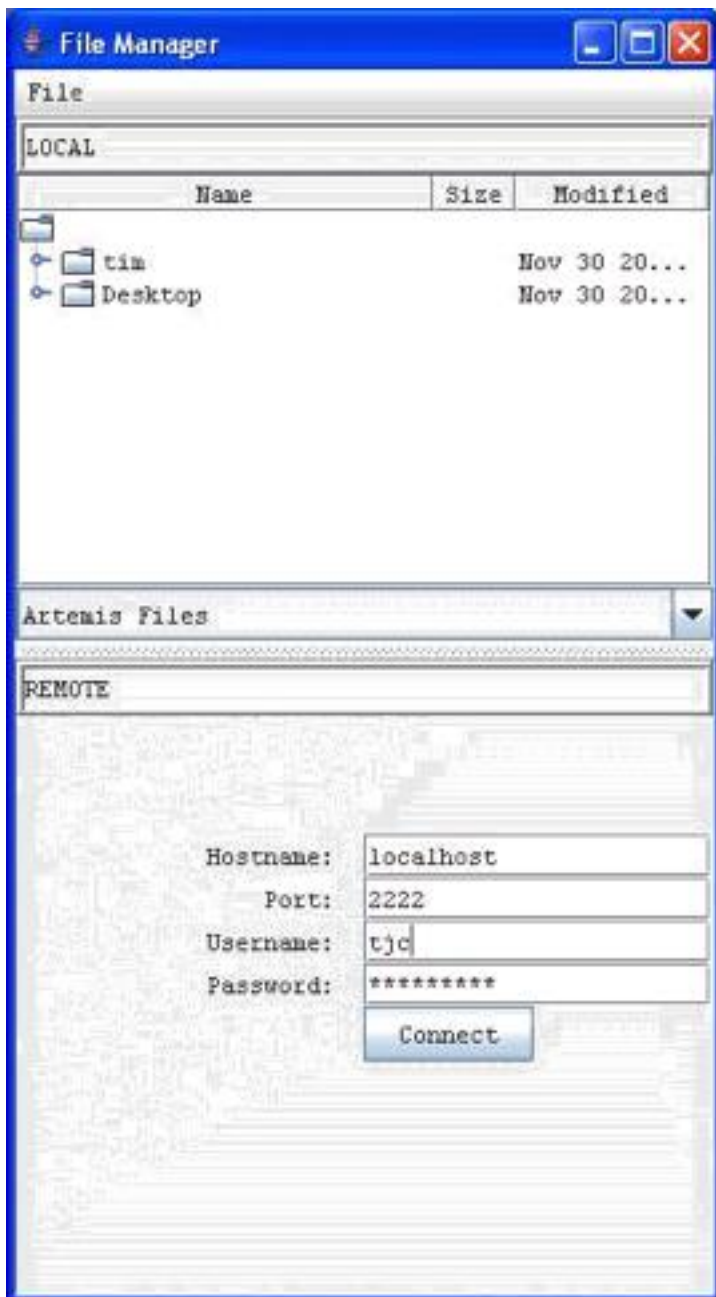
Set the source port to 2222 and set the destination to hostname:port (e.g. pcs2.internal.sanger.ac.uk:22).



Click 'Add' to add this to the list of forwarded ports.



Run Artemis and select the 'SSH File Manager' from the 'File' menu. At the bottom fill in login details and click on the 'Connect' button. Logging in may take a minute (depending on the connection).



Using the SSH connection to run Fasta and BLAST

Using the SSH connection you can send Fasta and BLAST searches to be run on the remote machine. If you have not already be prompted to login (i.e. when opening up the SSH file manager), you will then be asked for your login details. The sequence files that are to be searched against the database are then sent

to the remote machine. Once the search has finished the results are transferred back afterwards. If ACT detects that you have opened up an entry from the remote file system the analysis will be run in the directory you open the entry from. The results in this case are stored on both the local and remote machines.

To keep Fasta and BLAST searches on your local machine and the remote file system *in-sync*:

- copy any previously run fasta and blast directories from the remote machine to your local file system. This can be done by making a tar ball of the directory: e.g. creating a tar file of a 'fasta' directory

```
tar cvf tar_ball_name.tar fasta
```

- Then transfer this file to the local project directory and extract with:

```
tar xvf tar_ball_name.tar
```

- when launching Artemis, make sure you first change directory to the directory above the 'fasta' directory that has been unpacked.
- open the files from the remote file system in the file manager. It then knows where to save it back to.

Configuring Searches to be run via SSH

This would most likely be done by a systems administrator. The databases and fasta and BLAST software need to be set up on the remote machine. Once this is done the commands that are sent from the Artemis/ACT client to the remote machine are controlled by a 'j2ssh.properties' file, see below for example of this file. This needs to be put in the CLASSPATH for Artemis/ACT or the one in the distribution (lib/j2ssh/j2ssh.properties) modified.

The j2ssh.properties file also hold default information. For example default parameters can be set for the login prompt, the working directory (for searches when sequences are opened on the local side) and any extra directories (other than the users home directory) to display in the file manager.

```
#
#
# Configuration file for using SSH file management and
# database searches.
#
#
# default login
options port=2222
host=localhost
#
#
# Generic default options
#
blastp=blastall -p blastp
blastx=blastall -p blastx
```

```

tblastx=blastall -p tblastx
blastn=blastall -p blastn
fasta=fasta33_t -B -S -q -b 100 -H -l /data/fasta/pubseqgbs
fastx=fastx33_t -B -S -q -b 40 -H -l /data/fasta/pubseqgbs
#
#
# default working directory
wdir=/scratch
#
#
# aliases used for fasta
%uniprot=%U
%uniprot_archaea=%A
%uniprot_bacteria=%B
%uniprot_eukaryota=%E
%uniprot_viruses=%V
%uniprot_rest=%R
%malaria=%M %kineto_aa=%K
default_db=%uniprot_bacteri
a
#
#
# remote directories to use as roots in the file
manager remotedir.PD=/nfs/pathdata
remotedir.YP=/nfs/disk222/yeastpub
# local directories to use as roots in the file
manager localdir.PD=/nfs/pathdata
localdir.YP=/nfs/disk222/yeastpub

```

Chapter 5. ACT Configuration

Options The Options File

When started on ACT will potentially look in 5 different places for files to examine for configuration information. ACT reads from the locations in order, so the user can override the default options. Note that on Macintosh and Windows systems the "current directory" mentioned below is the directory where the ACT application has been installed.

This is the search order:

- The standard ACT options are read first. On UNIX this file is in the file `etc/options` in the ACT distribution directory. Changing that file will change the options settings for all users. On Macintosh and Windows system the standard options are stored in the ACT application itself and can't be changed (easily).
- If the user has specified an options file on the command line with the `-options` argument, it will be read next. (UNIX only - see [the section called UNIX Command Line Arguments for ACT in Chapter 2](#) for more).

- If there is a file called `diani.ini` in the current directory it will be read. This is for backwards compatibility.
 - If there is a file called `options`, `options.txt` or `options.text` in the current directory it will be read.
 - If there is a file in the user's home directory called `.artemis_options` it will be read.
-

ACT Option Descriptions

This section describes the possible options for ACT.

font_size

This option will set the font size for all the ACT windows. [default: 14]

font_name

This option is used to choose the font for all the ACT windows. The font must be fixed-width. [default: "Monospaced"]

base_plot_height

The height (in pixels) of each of the base plots. [default: 150]

feature_plot_height

The height (in pixels) of each of the feature plots. [default: 160]

draw_feature_borders

If set to yes, borders will be drawn around each feature and each exon. if set to no, borders will only be drawn around the selected features. This can also be set in the views popup menu (see [the section called *Feature Borders in Chapter 3*](#)). [default: "yes"]

show_forward_lines

If set to yes, the forward frame lines are shown by default. This can also be set in the views popup menu. [default: "yes"]

show_reverse_lines

If set to yes, the reverse frame lines are shown by default. This can also be set in the views popup menu. [default: "yes"]

draw_feature_arrows

If set to yes, a direction arrow will be drawn around at the end of each feature. if set to no, no arrows will be drawn. This can also be set in the views popup menu (see [the section called *Feature Arrows in Chapter 3*](#)). [default: "yes"]

overview_feature_labels

If this option is no then the feature labels in the overview (see [the section called *Overview of the Comparison Window in Chapter 3*](#)) will be off at startup. [default: "yes"]

overview_one_line_per_entry

If this option is set yes then the overview (see [the section called *Overview of the Comparison Window in Chapter 3*](#)) will start in one line per entry mode. [default: "no"]

show_list

If set to yes then the feature list (see [the section called *The Feature List in Chapter 3*](#)) will be shown on startup. [default: "yes"]

show_base_view

If set to yes then the DNA base view (see [the section called *The DNA Views* in Chapter 3](#)) will be shown on startup. [default: "yes"]

features_on_frame_lines

If set to yes then the "All Features On Frame Lines" option will be set to yes on startup. (See [the section called *All Features On Frame Lines* in Chapter 3](#)) [default: "no"]

frame_line_features

This is a list of the feature keys that should be shown by default on the frame lines. e.g.

```
frame_line_features = \  
  CDS \  
  polypeptide
```

feature_labels

If set to yes then the feature labels will be shown on startup. (See [the section called *All Features On Frame Lines* in Chapter 3](#)) [default: "yes"]

one_line_per_entry

If set to yes then the "One Line Per Entry" option will be set to yes on startup. (See [the section called *All Features On Frame Lines* in Chapter 3](#)) [default: "no"]

genetic_codes

This option gives the list of names of the available genetic code tables. For each name in the list there is a translation_table_NUMBER entry (see below) where NUMBER is its location in the genetic_codes list. Similarly the start codons are defined as start_codons_NUMBER for each code.

translation_table_1

The translation_table option is used to lookup codon translations. The table must have exactly 64 entries, and there is one entry for each codon.

The entries should appear in this order:

```
TTT TTC TTA TTG
TCT TCC ...
...
```

This is the default setting for this option is the Standard Code:

```
translation_table_1 = \
    f f l l \
    s s s s \
    y y * * \
    c c * w \
    \
    l l l l \
    p p p p \
    h h q q \
    r r r r \
    \
    i i i m \
    t t t t \
    n n k k \
    s s r r \
    \
    v v v v \
    a a a a \
    d d e e \
    g g g g
```

The other translation table entries are defined by giving the differences to the Standard Code. These are given as the bases that make up the codon immediately followed by the translation (e.g. atam, so that 'ata' codes for 'm').

extra_keys

This contains a list of keys (separated by spaces) that are allowed in addition to those specified by EMBL. The official EMBL keys are listed in the feature_keys file in the ACT code directory.

extra_qualifiers

This contains a list of qualifiers (and their associated type) that are allowed in addition to those specified by EMBL. The official EMBL qualifiers and qualifier types are listed and described in the `qualifier_types` file in the ACT code directory.

common_keys

This is a list of the keys that should be shown by default in the feature edit window. (see [the section called *Selected Features in Editor* in Chapter 3](#)).

The default setting for this option is:

```
common_keys = \  
  allele attenuator CDS conflict exon intron LTR misc_feature misc_RNA mRNA  
  \ mutation polyA_signal polyA_site promoter protein_bind RBS repeat_region  
  \ repeat_unit rRNA scrRNA snRNA source stem_loop STS TATA_signal terminator  
  \ tRNA unsure variation -10_signal -35_signal CDS_motif gene \  
  BLASTN_HIT CDS_BEFORE CDS_AFTER BLASTCDS
```

hyperlinks

This is a list of databases and their URL's for Artemis to provide hyperlinks in the Feature Editor (see [the section called *Selected Features in Editor* in Chapter 3](#)).

undo_levels

The number of levels of undo to save or 0 to disable undo. More undo levels will require more memory. [default: 20]

minimum_orf_size

This option is used to set the minimum size (in amino acid residues) of a "large" open reading frame, which controls which ORFS are marked by the "Mark Open Reading Frames" menu item (see [the section called *Mark Open Reading Frames ...* in Chapter 3](#)).

direct_edit

Set the default value for the direct edit option. A value of "yes" will turn direct edit on by default. See [the section called *Enable Direct Editing* in Chapter 2](#)

feature_dna_programs

This is a list of the possible external programs that can be run on the bases of a feature. Each pair in the list is a program name and a default database to use for that program. For each program name there must be a corresponding shell script called "run_something". eg. run_blastn. See [the section called *Configuring the Run Menu* in Chapter 3](#) for more information.

feature_protein_programs

This is a list of the possible external programs that can be run on the translation of a feature. Each pair in the list is a program name and a default database to use for that program. For each program name there must be a corresponding shell script called "run_something". eg. run_blastp for blastp or run_fasta for fasta. See [the section called *Configuring the Run Menu* in Chapter 3](#) for more information.

ncbi_dna_search

This lists the NCBI web-BLAST applications that the bases of a selected feature can be sent to. See [the section called *The Run Menu* in Chapter 3](#) .

ncbi_protein_search

This lists the NCBI web-BLAST applications that the translation of a selected feature can be sent to. See [the section called *The Run Menu* in Chapter 3](#) .

Setting Colours

The feature colours (see [the section called *Feature Colours* in Chapter 1](#)) used by ACT can be changed using the options file. By default there are 18 possible colours (numbered 0 to 17), but any number can be used. The option names for the colours are colour_0, colour_1, etc. The value of each of these qualifiers should be the three numbers separated by spaces. The numbers correspond to red, green and blue respectively and each number is an intensity from 0 to 255. As an example, to change colour 2 to white put this line in the options file:

```
colour_2 = 255 255 255
```

Here is a list of the default colour numbers:

0	white	(RGB values: 255 255 255)
1	dark grey	(RGB values: 100 100 100)
2	red	(RGB values: 255 0 0)
3	green	(RGB values: 0 255 0)
4	blue	(RGB values: 0 0 255)
5	cyan	(RGB values: 0 255 255)
6	magenta	(RGB values: 255 0 255)
7	yellow	(RGB values: 255 255 0)
8	pale green	(RGB values: 152 251 152)
9	light sky blue	(RGB values: 135 206 250)
10	orange	(RGB values: 255 165 0)
11	brown	(RGB values: 200 150 100)
12	pale pink	(RGB values: 255 200 200)
13	light grey	(RGB values: 170 170 170)
14	black	(RGB values: 0 0 0)
15	mid red:	(RGB values: 255 63 63)
16	light red	(RGB values: 255 127 127)
17	pink	(RGB values: 255 191 191)

Options For Plots and Graphs

Each graph type has three option settings associated with it: the default minimum window size, default maximum window size and the default window size. The option names have the following form: [short_name]_default_min_window, [short_name]_default_max_window and [short_name]_default_window (respectively). [short_name] should be replaced with the short name of the graph. (The available short names are documented in [the section called *The Graph Menu* in Chapter 3](#) and [the section called *Feature Plots* in Chapter 3](#)).

As an example the short name of the GC content graph is gc_content so to set the initial minimum window size to 100, the maximum to 1000 and the initial window size to 150 use these setting:

```
gc_content_default_min_window = 100
gc_content_default_max_window = 1000
gc_content_default_window_size = 150
```

See also [the section called *Graphs and Plots* in Chapter 3](#) .

Options For A Chado Database

chado_exon_model

This is the feature key used when chado exons are joined and displayed in ACT. For example:

```
chado_exon_model=CDS
```

[default: "exon-model"]

chado_transcript

This is the feature key used when chado transcripts are created in a gene model. [default: "mRNA"]

chado_servers

This can be used to provide a list of available databases. For example:

```
chado_servers = \  
    test localhost:5432/test?userName \  
    genedb_ro db.genedb.org:5432/snapshot?genedb_ro
```

Options File Format

The option files it should contain settings that look like this:

```
option_name = option_value
```

If the value of an options is too long to fit on one line it can be split over several lines by ending each line with a backslash like this:

```
option_name = option_value another_option_value \  
    a_third_option_value a_forth_option_value
```

An Example Options File

Here is an example options file:

```
# this line is a  
  
comment font_size = 14  
  
minimum_orf_size = 100  
  
common_keys = \  
    allele attenuator CDS conflict exon intron LTR misc_feature misc_RNA mRNA  
    \ mutation polyA_signal polyA_site promoter protein_bind RBS repeat_region
```

```
\ repeat_unit rRNA scRNA snRNA source stem_loop STS TATA_signal terminator  
\ tRNA unsure variation -10_signal -35_signal CDS_motif gene
```